U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-280 565

Journal Articles on Toxicology. Group 12

Environmental Protection Agency, Washington, D C

1975

PB 280 565

Mother Sheet

Title:

Journal Articles on Toxicology. Group 12.

PB 280 566

Reprinted from Baculoviruses for Insect Pest Control: Safety Considerations
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Guidance for Safety Testing of Baculoviruses

U.S. Environmental Protection Agency

The following guidance summarizes the data presently required by the Agency to show the safety of nuclear polyhedrosis and granulosis virus insecticides. (These two groups of insect viruses in the following are referred to as "virus" or "insect virus.")

When testing the safety of insect viruses, primary emphasis must be given to their pathogenicity and not to their toxicity. Pathogenicity is the capability of the microorganism to infect or reproduce, or to cause overt or covert changes, in animals or animal tissue other than target insects. Toxicity means that microorganisms, and/or their by-products, harm the test animal without actual multiplication such as in certain adverse immunological reactions.

Experiments must be designed to support the assumption that the insect pathogen is not a pathogen for humans, other vertebrates, and nontarget invertebrates, nor a hazard to the environment. Proving this hypothesis unequivocally is impossible; however, the required tests cover such a multitude of different approaches that the results will permit an evaluation of the safety of an insect virus and a prediction of the possible hazards.

The safety evaluation studies must be done under the guidance of qualified scientists who, by training and experience, can plan and employ scientifically sound experimental procedures and can assess toxicological, infectious, and pathological manifestations. Any insect virus that causes adverse effects related to overt or covert infection or reproduction in nontarget test animals should not be considered as a potential control agent. Nothing in this guidance shall be construed as relieving the investigator of these responsibilities.

The requirements for safety testing depend on the proposed uses of the virus. The impact of the virus on the environment and other organisms must be considered. In specified instances and with limited use, a temporary exemption from the requirement of a tolerance is granted in accordance with an experimental use permit for experimental field trials of the virus. Generally, and when used on an experimental basis on food crops, the acute and subacute feeding studies must be completed, as well as eye and dermal irritation/toxicity tests, with the virus and the formulation. Depending on the type of use and application, inhalation studies also must be completed. The insect host spectrum of the virus must be studied. The possible effects on fish, birds, and invertebrates (other than insects) must be determined. If the registrant feels that any of these requirements should be waived for uses on nonfood crops (and possibly food crops), an argument for waiver must be presented.

The virus preparation used for safety testing must be the same technical product as that which is subsequently produced and used commercially. The virus must not be inactivated by components of the diet with which it is fed to test animals. Certain safety tests, such as eye and skin irritation tests and acute exposure feeding tests, may also be required for the formulation, especially in the case where materials not previously tested and approved are used in the formulation.

The virus must be an insect pathogen which is identified taxonomically according to host spectrum and serological, biochemical, and biophysical tests. The fungal and bacterial contamination of the technical virus due to production procedures must not exceed the limits which can be maintained under sanitary manufacturing conditions. More importantly, human and mammalian pathogens (e.g., *Salmonella*, *Shigella*, and *Vibrio*) and microorganisms producing toxins known to affect mammals must be absent from the technical products.

Quantitation of virus must be based on bioassays. Standardization of bioassays is difficult; the variability of the assay, however, should be known.

Since the following guidance is subject to revision according to the most recent knowledge about viral insecticides, it is recommended that petitioners confer with Agency officials before starting the tests described below.

Protocols should be designed so that the data can be evaluated statistically. The actual number of test animals to be used is generally not specified in order to permit the most individualized approach to each test. Where numbers are indicated, they are meant as suggested minimal numbers.

SAFETY TESTS

A. Dosage

Since the concepts of no-effect level and LD_{50} for species other than the natural hosts cannot be applied to insect viruses, the following procedure must be used to determine oral dosages. The dosage calculated below is arbitrary; it will, however, introduce a liberal margin of safety since an

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individual will very likely never be exposed to the quantity of active virus applied to 100 acres. Introduction of lower dosage levels will not provide additional knowledge about the possible pathogenicity of the virus.

The quantity of biologically active virus expected to be applied to 100 acres is determined. In designing the laboratory animal experiments, it is assumed that a 70-kg man would be exposed to this quantity, and the presumed 100-acre equivalent exposure of a man is calculated in active virus per kilogram of body weight. The experimental animals are then exposed to the same dose adjusted to their weights. In acute tests, the entire amount is given as a single dose; in 90-day feeding studies, the total quantity is fed in 90 portions. If the determined amount of virus is excessive, especially for a single dosage, a 10-acre equivalent can be used. For a single dose, the amount of virus should not exceed 50% of the daily food intake of the experimental animal; for multidose experiments, the addition of virus to the diet should not interfere with the nutritional needs of the test animals.

The above dosage is meant for feeding studies and with technically necessary adjustment for dermal exposure studies. For dosages used in other tests, such as inhalation and tissue culture experiments, one should refer to the respective sections below.

B. Single-dose exposures

1. Feeding studies

a. Schedule of feeding and sacrifice. The insect virus is administered in a single dose to young adult laboratory animals of both sexes (two species). Some animals (at least one of each sex) should be sacrificed shortly after feeding of the virus, and others should be sacrificed later, at intervals of hours to several days, until the termination of the experiment. Shorter intervals should be used at the beginning of the test. Some animals (at least three of each sex) should be left to survive for at least 35 days to make serological tests meaningful. Animals (one mammalian species) which are on an effective regimen of immunosuppressive drugs must be included in this study (one control group and one group which is exposed to virus).

b. Data to be collected from experimental animals.

i. Growth, food consumption, general appearance, mortality, and morbidity.

ii. Attempts to isolate and detect insect virus inclusion bodies or virions in gastrointestinal tract, blood, urine, and selected body tissues which would normally be affected by a viral infection (e.g., lymph nodes, kidney, cerebrospinal system, spleen, liver). Use the most specific and sensitive assay for virus detection.

iii. Body temperature, measured twice daily at the same times each day.

iv. Antibody response to viral proteins.

v. Hematology, urinalysis, organ function tests, organ weights, and gross and microscopic pathology. It will suffice to investigate these items at the end of the experiment and possibly at the midterm.

vi. All animals dying before termination should be examined grossly and microscopically, and the cause of death should be determined, especially if a microbial etiology is suspected.

2. **Eye irritation study.** Usually performed on six albino rabbits; observation after 1, 2, and 3 days, and longer if irritation persists.

3. **Dermal toxicity and skin irritation.** Single application of insect virus to intact and abraded skin (rabbit) with observation for 21 days; necroscopy of animals after 21 days, with gross pathology and histological examination in cases where pathological changes of skin or organs are noted.

4. **Inhalation study.** Administration of virus product as dust or aerosol, by single exposure. The concentration of the aerosol and the duration of exposure must be such as to reflect an unusually high exposure level of a man using the insecticide spray. Shortly after exposure and at intervals thereafter, the lung and other tissues of the respiratory tract must be assayed for the presence of virus. The residual virus must be isolated from

the respiratory tract by bioassaying, with a sensitive insect larval test, tissue, and aspirated pneumobronchial washings. Some animals must be observed for at least 2 weeks, after which time a terminal necroscopy with gross pathology and histological examination of the respiratory system must be performed.

5. **Human, primate, and nonprimate tissue culture studies.** Tissue culture studies, though not representative of the conditions of the intact animal body, must be used to explore the safety of insect viruses, i.e., the absence of viral multiplication, inapparent association with the cell, or other interference at the cellular level. Primary or other diploid cells of human, primate, and nonprimate origin should be exposed to purified, infectious virus (free from insect fragments and bacterial contamination).

The optimal conditions, such as multiplicity of infection, buffer system, optimal temperature for adsorption and penetration, for infecting tissue cultures must be determined using host insect cells. The homologous tissue culture system must show a successful infection and will serve as positive control. If possible, the infectious unit(s) should be isolated and identified. In cases where homologous insect tissue cultures are not yet available for testing the infectivity of the inoculum, the vertebrate cells should be challenged with infectious hemolymph, because it presumably contains infective units capable of infecting tissue culture cells.

Some duplicate cultures of the exposed vertebrate cells also must be maintained at lower-range temperatures (about 30 C, or lower if possible) to assess the possible interactions of insect virus under conditions which approach the optimum for its invasion, multiplication, and interactions in living insects or insect tissue cultures.

Techniques of transfection (forced entry and interaction of viruses and virus genomes with nonpermissive cells) also can be applied; for more detail, refer to the section on long-term studies.

Observations. For cytopathic effects and the formation of inclusion bodies in the living and fixed and stained cells, but also for more subtle changes such as cell transformation and interference with viruses which would normally grow in the tissue cultures.

Three blind passages must be performed in each cell type, and each passage should be maintained as long as possible, but at least 14 to 21 days. At each passage level, a chromosome analysis of the exposed and control tissue culture cells must be performed. The last passage also must be tested by quantitative bioassay in susceptible insect larvae to determine whether multiplication of infectious insect virus (inclusion bodies or virions) occurred in the tissue cultures.

The fluorescent-antibody technique, for example, or other sensitive methods must be applied to a selected number of exposed tissue cultures, to determine whether viral antigens or subunits, which would not be detected by bioassays, were produced.

C. **Multiple-dose exposure.**

1. **Oral exposure by feeding or by direct dosing.**

   a. **Number of species.** At least two, one a nonrodent.

   b. **Duration.** Ninety days.

   c. **Observations.** Growth, food consumption, general appearance, mortality, morbidity, hematology, urinalysis, organ function tests, organ weights, gross and microscopic pathology. Tissues examined should be those described in the WHO Technical Report Series No. 426 (1969), with special emphasis on those expected to be affected by viral infections (intestine, nervous tissue, lymphatic tissue, spleen, liver, kidney). All animals dying before termination should be examined grossly and microscopically as well as for microbial organisms which might be the etiological agents of death. The antibody (circulating) response of a sufficient number of animals should be studied after 30, 60, and 90 days.

   Several virus diseases of vertebrates are known where the fetus appears to be the most affected target. These diseases also often show no or minimal clinical signs in the adult. The search for a teratogenic effect after viral

exposure per os, during the gestation period, therefore, could be a very sensitive method for the detection of an adverse effect on the adult (female).

2. **Skin sensitization.** Multiple intradermal injections of virus material and/or patch application. For allergenicity tests, guinea pigs should be used preferentially as test animals. Dosing and observation for 3 weeks with challenge injections 3 to 7 days later to assess sensitization. In case of extreme sensitization, the antibody response of the animals should be investigated.

3. **Respiratory sensitization and inhalation toxicity.** Guinea pigs must be exposed to dust or aerosols of the insect virus for 1 h each, four or five times per week for 3 weeks. One week after the last exposure, the animals are challenged by the respiratory and dermal route to assess allergic reactions. At termination of the experiments, the animals are also investigated for pathological changes of the pneumobronchial system.

D. **Long-term studies.**[1] The present knowledge concerning the interaction of insect virus with permissive and nonpermissive cells does not allow a final conclusion on whether or not an interaction at the molecular or genetic level is possible. Such interactions would most likely manifest themselves in long-term studies and not in acute and subacute ones. It is therefore proposed that a two-generation lifetime oral exposure study be conducted to assess possible effects on animals exposed to insect viruses from the time of their conception until the end of their life span. The study will be carried out according to standard protocols for long-term carcinogenicity studies.

The basic question, can or will insect viruses interact with vertebrate cells, still remains unanswered. If it could be answered in the negative, carcinogenesis, mutagenesis, and reproduction studies could be omitted. The following approach to resolve the question is suggested. (The important aspect of the approach is that specific and sensitive detection methods for molecular or genetic interactions are used. Such methods have not been developed or applied to insect virus systems.)

1. The invertebrate cell-virus system must be defined as a control. The uptake and behavior, such as uncoating, and the growth curve and maturation of the virus and virus components must be studied. The infectious unit or units must be defined.
2. Human, vertebrate, and invertebrate cells should be challenged using the conditions found most suitable for the permissive control system outlined under 1.
3. Artificial methods should be used to get infectious units into nonsusceptible cells, such as DEAE-dextran, solid carriers (kaolin, bentonite), and osmotic or nutritional manipulation. After both the simple and artificial challenge of cells, observations must be made for overt infection, persistent infection, or inapparent infection. In order to identify an inapparent infection, an attempt should be made to detect cell surface changes (antigens) and intracellular soluble antigens, for example. Viral interference tests and observation for morphological changes also would indicate the presence or absence of inapparent infections. DNA hybridization may be another suggested but untried method to detect subinfectious interaction.

E. **Beneficial insect, wildlife, and plant toxicity.** The following toxicity tests are designed to assess the possible adverse impact on nontarget invertebrate and vertebrate species and plants exposed to virus in the environment. The dose must reflect the level of insect virus to which the environment is exposed under the expected conditions of virus insecticide use (for example, infectious virus units per liter of water or per square meter of forage area). A level 100 times this and one intermediate level must also be tested. Some acute studies customarily required for the assessment of fish and wildlife toxicity seem of little value in the case of insect viruses; subacute studies will give more assuring results.

1. **Effects on invertebrates.** The host spectrum of the insect virus must be deter-

[1] The purpose of long-term studies is to help in the prediction of possible effects on man or the environment resulting from prolonged (life-long) low-level exposure, such as intake via food. Since insect viruses are a natural component of the environment, it is impractical to be concerned about exposure to natural levels of virus. Quantitative determinations of virus levels (see section F, subsection 2) thus may be sufficient to determine whether or not a possible long-term hazard from the use of insect viruses exists, especially in those cases where the use of viruses does not significantly increase, or even decreases, the level of exposure.

mined (see identification of pathogen). Honeybees and other beneficial or aesthetically and commercially important invertebrates, as well as parasites and predators of the target insect, should be included in the experiments which determine the host range. Data for evaluating the effects on nontarget insects can be obtained through laboratory experiments as well as field sampling procedures.

2. **Effects on fish (and other aquatic vertebrates).** Representative fish species must be exposed to insect virus. The virus must be added in suspension to the water and in the form of diseased larvae to be eaten by insectivorous fish. The period of exposure and observation must be extended to 30 days, after which time an autopsy of the fish should be performed.

   An alternative or extension of the fish exposure studies would consist of exposing fish and amphibian tissue culture to virus. The problems of detecting virus interaction at the cellular level are pointed out in the section on mammalian tissue culture studies and long-term studies. These same problems apply to fish tissue cultures and must be carefully considered by the investigator.

3. **Effects on birds.** Two species of birds, including at least one insectivorous species, must be exposed to virus by the oral route. Since birds may act as a vector of insect viruses via their droppings, the fate of the virus in the birds' digestive tracts must be studied. Birds, in fact, may be included in the acute studies listed under B.1 or C.1 since some species are expected to eat large quantities of virus when they feed on diseased insects.

   Bird reproduction studies are not required; the Agency, however, retains the option to require such studies in case a review of data indicates that a problem may exist.

4. **Effects on plants.** No controlled studies to investigate effects on plants are needed. During experimental use, however, protocols must be designed to detect phytotoxic effects, should they exist. The finding of these observations must be reported.

F. **Monitoring.** Monitoring of effects and impacts of pesticides is an important aspect of their overall safety. Logically, monitoring cannot be a prerequisite for experimental use; rather, it must be undertaken during experimental use and extended to periods of full use. Continued monitoring of the environment and exposed humans will provide additional assurance that a virus which has undergone safety tests elicits no long-term adverse effects which might have escaped observation. Effective monitoring depends on the development of sensitive and specific assay techniques which presently are not completely finalized. The following, therefore, is a suggested and possibly crude approach which should be changed once the necessary experience has been gained.

1. **Human monitoring.** Human exposure experiments are not required. However, a research or industrial team working with potential virus insecticides provides an excellent opportunity to check the responses (especially serological and allergic responses) of exposed humans. Surveys and long-term follow-up studies on such occupationally exposed persons are, therefore, highly recommended. Presently, only general health surveys including neurological function tests can be suggested. In cases where specific serological tests are available, sera of exposed humans must be assayed; where tests are not yet available, sera should be stored frozen for later use. It is important that a series of, serum samples of each individual be available.

   Prospective and retrospective epidemiological studies of high-exposure groups may also aid in the assessment of possible ill effects on humans.

2. **Environmental monitoring.** The most important aspect of environmental monitoring is an investigation of virus levels in nature (host plants of target insects, water, etc.) and the development of techniques and methods to perform this type of monitoring activity. A variety of conditions may exist and have been documented; for example, epizootics in forest insects produce hundreds of times the amount of virus usually applied to forests, and virus levels on food crops may be less after purposeful introduction of the virus than after natural epizootics late in the growing season. The assessment and monitoring of "natural" virus levels and virus levels after use in relation to the amount applied are very important aspects of the safety evalua-

tion. Observation for unusual ecological occurrences in treatment areas may be another empirical approach to environmental monitoring. Systematic assessment of wildlife species, including blood collection of hunted species for serological tests, should also be undertaken.

G. **Changes and/or additions of experimental designs.** The proposed safety testing experiments for insect viruses are still in need of improvement. Quantitative virus isolation, serological tests, biochemical-biophysical virus identification, and specific detection methods at the cellular level are some of the areas where progress is needed. Therefore, it is the duty of every scientist as well as Agency official involved in this problem to contribute to the improvement of safety testing, and suggestions based on scientific evidence are needed. Changes resulting from new scientific data should be submitted to the Agency and discussed with Agency officials to assure that any new approach satisfies the Agency's requirements.

Administration of virus to animals via injection is not strictly required, since such procedures have little semblance to natural exposure patterns except in cases where biting insects are to be controlled. Tests using parenteral administration of virus, however, are highly recommended, and they could be combined with the needed antiserum production. The criteria for monitoring the animals and the fate of the virus should closely follow the criteria listed under acute feeding studies.

August 1975

PB 280 567

Biochemical Pharmacology, 1963, Vol. 12, pp. 1377–1386. Pergamon Press Ltd., Printed in Great Britain.

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TOXICOLOGY PROGRAM
Technical Information Branch
Communicable Disease Center
Atlanta 22, Ga.

RELATIONSHIP BETWEEN DEPRESSION OF BRAIN OR PLASMA CHOLINESTERASE AND PARALYSIS IN CHICKENS CAUSED BY CERTAIN ORGANIC PHOSPHORUS COMPOUNDS*

ROBERT F. WITTER and THOMAS B. GAINES

Toxicology Section, Communicable Disease Center,
United States Public Health Service, Atlanta, Ga., U.S.A.

(*Received* 17 *May* 1963; *accepted* 22 *July* 1963)

Abstract—A study has been made of the relationship between the inhibition of plasma or brain cholinesterase of the chicken and the paralytic syndromes caused by DFP, TOCP, malathion, ronnel, EPN, and Trithion. DDVP and Dipterex served as non-paralytic controls. TOCP was the only compound tested that was a specific inhibitor of brain cholinesterase. All compounds except EPN inhibited plasma cholinesterase. All compounds tested inhibited brain true and/or pseudocholinesterase. All paralytic compounds tested except DFP produced a prolonged inhibition of brain true and/or pseudocholinesterase which lasted for 5 to 17 days. It was concluded that no causative relationship exists between the prolonged inhibition of brain cholinesterase and the paralytic syndromes. The regeneration of brain cholinesterase after inhibition with DFP, Dipterex, or DDVP is probably due to protein synthesis *de novo*; the prolonged inhibition of these enzymes after the administration of the other compounds may be due in part to the persistence of the inhibitor in the body.

THE PARALYTIC effect of certain organic phosphorus compounds on chickens has been used as an index of possible paralytic action in man, since TOCP[1, 2] and mipafox,[3, 4] which produce paralysis in man, were found to produce a similar syndrome in chickens. As far as we are aware, no clear-cut cases of paralysis in man have been reported in connection with other paralytic compounds, even though human cases of anticholinesterase poisoning by DFP, malathion, and Trithion are known. It is true that there have been reports of temporary paralysis in five people exposed to malathion,[5–8] but the cases varied widely among themselves and occurred in people with little exposure rather than in people with massive exposure, including those poisoned by ingesting the compound.

The paralysis produced in chickens with malathion and some other organic phosphorus derivatives differs from that noted with TOCP, mipafox, or DFP in time of onset after dosing and/or in duration.[9] The most outstanding difference between the two groups of paralytic compounds is that the syndrome does not appear until about 10 to 14 days after dosing with DFP, TOCP, or mipafox,[9–14] whereas with EPN and malathion, the syndrome usually develops within 1 or 2 days.[9]

A number of investigations have been made on the effects of the neurotoxic organic phosphorus compounds on the brain and plasma cholinesterase of the chicken, but these have been restricted to TOCP,[11, 13–17] its metabolites[17] and similar aromatic

* See Table 1 for chemical names of compounds and abbreviations.

phosphates,[18] to mipafox,[15, 16] and to DFP.[15, 16] In these studies no definite correlation was found between inhibition of cholinesterase and paralytic effects, although each compound inhibited plasma or brain pseudocholinesterase *in vivo*.

However, in view of the two kinds of paralytic syndrome, it seemed worthwhile to study the effects on brain and plasma cholinesterase activity of compounds with immediate, temporary neurotoxicity in an attempt to determine whether the effects of these substances differ from the effects of TOCP, mipafox, DFP, or from nonparalytic compounds. In connection with this study, preliminary tests were conducted on the neurotoxicity of some organic phosphorus compounds not previously reported, including Dipterex, ronnel, and Trithion.

In these experiments, EPN, malathion, ronnel, and Trithion were studied as representative of the compounds that may produce paralysis within 1 or 2 days after dosing. TOCP and DFP were investigated as representative of those compounds that produce paralysis after a delay of 10 to 14 days. The studies with the latter two compounds permitted direct comparison of the results of the present investigation with those of other workers. Dipterex and DDVP were the two nonparalytic compounds investigated.

TABLE 1. COMMON AND CHEMICAL NAMES, GRADE, AND SOURCE OF SUPPLY OF COMPOUNDS TESTED*

Common name	Chemical name	Purity and grade	Source of supply
DDVP	O,O-dimethyl 2,2-dichlorovinyl phosphate	98% Refined	Montrose Chemical Co.
DFP	Diisopropyl fluorophosphonate	99%	Aldrich Chemical Co.
Dipterex (Dylox)	Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate	50%	Chemagro Corp.
EPN	O-ethyl O-*p*-nitrophenyl phenylphosphonothioate	Technical	E. I. DuPont Chemical Co.
malathion	O,O-dimethyl S-(1,2-dicarbethoxyethyl) dithiophosphate	Refined Practical 98%	American Cyanamid Co.
ronnel	O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothioate	100%	Dow Chemical Co.
TOCP	Triorthocresol phosphate	Practical	Eastman Kodak Co.
Trithion	O,O-diethyl *p*-chlorophenylmercaptomethyl dithiophosphate	95% Technical	Stauffer Chemical Co.
	Atropine sulfate		Merck and Co.
	Acetyl β-methyl choline bromide		K and K Laboratories
	Butyrylcholine chloride		K and K Laboratories
2-PAM iodide	Pyridine-2-aldoxime methiodide		Chemical Procurement Co.

* The use of trade names is for identification only and does not constitute endorsement of these products by the Public Health Service.

METHODS

The chemical names, grade or purity, and source of supply of the compounds used in these studies are shown in Table 1.

Butyrylcholine perchlorate was prepared by a modification of the method of Bell and Carr[19] from butyrylcholine chloride, and was recrystallized twice from cold ethanol. All the salts of choline esters, with the exception of butyrylcholine chloride, evolved the theoretical amount of CO_2 when hydrolyzed in the Warburg apparatus in

bicarbonate buffer with brain homogenate as described in the next section. All inorganic chemicals were of a reagent or analytical grade.

The procedure for carrying out the preliminary tests of the neurotoxicity of Dipterex, ronnel, and Trithion was essentially the same as that described by Durham *et al.*[9]

The chickens used were females of a sex-linked strain and weighed 2·5 to 3·0 kg. They were dosed subcutaneously on the right side between the thigh and the wing with the organic phosphorus compound in peanut oil, or with peanut oil alone if in control groups, after oral administration of atropine 15 to 20 min previously at a level of 15 mg/kg. The single exception was that Dipterex, because of its poor suspensibility in peanut oil, was given as an aqueous solution. Atropine was given in order to minimize the acute effects of the toxicant. The animals were observed for symptoms and each day tested for their ability to stand or walk on bare ground as previosuly described.[9] At various intervals, individual animals used in the study of cholinesterase inhibition were bled with heparinized syringes from the wing vein and then sacrificed by decapitation.

Twenty per cent (w/v) brain homogenates were prepared from whole chicken brains in ice-cold 0·15 M sodium chloride with an ice-cold motor-driven glass homogenizer of the type described by Dounce and Beyer.[10]

Cholinesterases were assayed in plasma or homogenates of brain by the Warburg manometric procedure in bicarbonate carbonic acid buffer under conditions similar to those described by Earl and Thompson.[21] The reaction mixture for the assay of plasma cholinesterase contained 0·035 M butyrylcholine. The plasma cholinesterase was corrected for the nonenzymatic hydrolysis of butyrylcholine (0·6 μmoles/30 min at 37°). Brain pseudocholinesterase was measured at 25° with 0·035 M butyrylcholine, and brain true cholinesterase was estimated with 0·040 M acetyl-β-methyl choline at 25°.

TABLE 2. THE NEUROTOXIC EFFECT OF ORGANIC PHOSPHORUS COMPOUNDS GIVEN SUBCUTANEOUSLY IN PEANUT OIL TO ATROPINIZED FEMALE CHICKENS

Compound	Dosage range tested (mg/kg)	Lowest dosage producing		Duration of paralysis (days)	Approximate subcutaneous LD_{50} (mg/kg)
		Cholinergic signs (mg/kg)	Paralysis (mg/kg)		
Dipterex	50–800	50			125*
ronnel	100–1,600	1,600	1,600†	4–24	> 1,600
Trithion	10–640	640	640	15–49‡	640

* Dosage levels of 200 mg/kg or greater killed all the chickens tested.
† Paralysis in 5 of 8 chickens tested at this dose, but none died.
‡ All 12 chickens dosed at this level developed paralysis. It was delayed 2 days in 4, 3 days in 1, 4 days in 3, and 7 days in 1 chicken(s).

RESULTS AND DISCUSSION

The results of basic studies establishing the neurotoxicity of Dipterex, ronnel, and Trithion in atropinized hens are shown in Table 2. The results for Trithion confirm the work of Edson, as reported by Hearn.[22] The results of similar studies carried out with DDVP, EPN, malathion, and TOCP were reported previously.[9]

Summaries of the symptoms produced in the chickens by the compounds, and of the changes in cholinesterase activities after dosing, are given in Tables 3 and 4. The effect

TABLE 3. PARALYTIC SYNDROMES FOLLOWING THE ADMINISTRATION OF CERTAIN ORGANIC PHOSPHORUS COMPOUNDS TO ATROPINIZED CHICKENS

The syndrome of immediate paralysis is defined as a muscle weakness that lasts 3 or more days so that the leg weakness noted 1 or 2 days after dosing with Dipterex, DDVP, and DFP is not considered to be the syndrome.*

The data in this table are summarized from the results of 5 separate experiments, each of which had its own control group and in which chickens were sacrificed for cholinesterase assay. The mortality data in this table include only those chickens that died spontaneously. The determination of the length of time the paralytic syndromes lasted is based on those chickens sacrificed last.

Compounds	Weight loss	Paralytic syndrome
Paralytic compound (delayed paralysis)		
TOCP 400 mg/kg	Large	No apparent symptoms until 12th day; no chickens died; then paralysis, starvation, weight loss
DFP 0·9 mg/kg	None	Six of 10 chickens died in 1 or 2 days; the others showed cholinergic symptoms and muscle weakness 1 or 2 days, recovery, and then developed paralysis on the 11th day after dosing
Paralytic compound (immediate paralysis)		
malathion 1,000 mg/kg	Moderate	Paralysis from 1st to 9th day; 5 of 16 chickens died 1 or 2 days after dosing; diarrhea for 3 to 4 days after dosing
EPN 60 mg/kg	Very large	Paralysis from 1st day to end of experiment; 4 of 13 chickens died between 11th and 29th day; ate very little
ronnel 1,600 mg/kg	Moderate	Paralysis from 1st or 2nd day to 7th day; no chickens died
Trithion 640 mg/kg	Large	Paralysis from 1st or 2nd day that lasted 17 days; no chickens died; ate very little; some diarrhea
Nonparalytic compound		
DDVP 18 mg/kg	Small	Paralysis for 1 or 2 days after dosing; 4 of 13 chickens died
Dipterex 90 mg/kg	Small	Paralysis for 1 or 2 days after dosing; 1 of 8 chickens died

TABLE 4. A COMPARISON OF THE CHOLINESTERASE LEVELS, 4 TO 16 DAYS AFTER DOSING, IN CHICKENS GIVEN THE NONPARALYTIC COMPOUNDS WITH LEVELS IN THOSE GIVEN THE PARALYTIC COMPOUNDS

The data are expressed as per cent of the average cholinesterase levels of the control chickens and are derived from the data in Figs. 1 through 3

Compound	No. of chickens	Days after dose	Per cent of control cholinesterase* Plasma Range	Plasma Mean	Brain true Range	Brain true Mean	Brain pseudo Range	Brain pseudo Mean
Nonparalytic compounds								
DDVP	4	4–14	64–80	74†	49–71	58	37–73	53
Dipterex	3	7–15	60–78	66	56–76	68	53–63	57
Paralytic compounds								
DFP	3	4–15			51–81	61	51–70	59
TOCP	6	5–16	24–46	32	55–78	69	25–39	33
malathion	5	4–14	80–107	95†	18–39	29	56–70	66
EPN	4	4–14	74–158	126†	9–22	16	41–77	65
ronnel	3	7–15	40–61	52	18–41	29	34–54	46
Trithion	3	7–15	15–26	20	9–18	13	19–33	27

* The number of control animals was equal to the number of experimental animals listed for each compound.

† Not statistically significant. Other values significant at 95% level or higher.

on brain and plasma cholinesterase (expressed as per cent of normal) for the nonparalytic compounds, for those that produced the delayed syndrome, and for those that caused paralysis without delay are summarized in Figs. 1, 2, and 3, respectively.

In confirmation of previous work it was found that DFP [15, 16] and TOCP[11, 14–16] produced paralysis 11 to 12 days after dosing, whereas a neurotoxic effect was noted within a short time after dosing with malathion and EPN.[9] No paralysis occurred after DDVP[9] was administered. The preliminary studies summarized in Table 2 were also confirmed in the separate tests to study cholinesterase activity. No correlation between structure of compound and paralytic effect was detected.

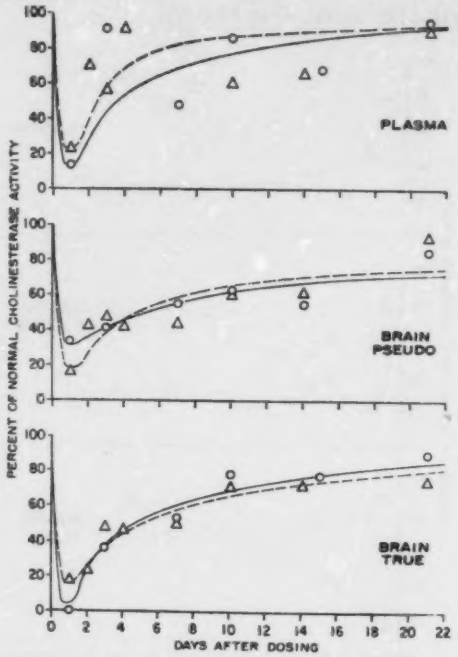

FIG. 1. Effect of DDVP (△) and Dipterex (○), nonparalytic compounds, on the plasma and brain cholinesterases of the chicken. The results are expressed as percentage of the cholinesterase activity found in a control chicken killed on the same day. Each point is calculated on the basis of one chicken in the experimental and control groups respectively.

As shown in Tables 3 and 4 and Figs. 1 through 3, all the organic phosphorus compounds tested in this report inhibited chicken plasma cholinesterase *in vivo* with the exception of EPN, a paralytic compound.

The effects of DFP on this enzyme were not investigated in the present studies, but Davison[15, 16] has shown that this organic phosphorus derivative does indeed inhibit this enzyme. The present results do not confirm the general rule that paralytic compounds inhibit plasma cholinesterase.[11, 13, 14, 16] Furthermore, no correlation was found between the degree or duration of the inhibition of this enzyme and the neurotoxicity of the compounds. The amount of inhibition of the plasma cholinesterase

and its rate of recovery did not necessarily parallel effects noted with the brain enzymes.

All the organic phosphorus compounds tested inhibited brain true and/or pseudocholinesterase. In confirmation of previous reports,[13, 17, 23] TOCP was found to be a more or less specific inhibitor of brain pseudocholinesterase, as shown in Table 4 and Fig. 2. However, this was not true with the other compounds because the level of brain true cholinesterase also was decreased to 10–20 per cent of the control level. The results obtained with DFP agree with those published previously.[15, 16] Thus it appears that specific inhibition of brain pseudocholinesterase is not characteristic of the paralytic compounds whether they are of the type that produces an immediate paralysis or a paralysis after about 10 to 14 days.

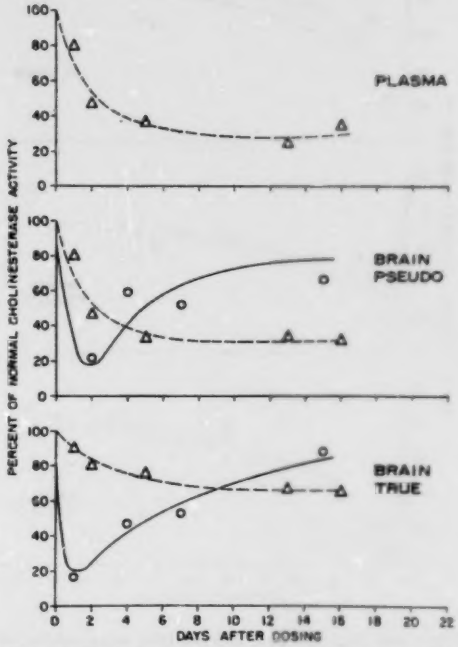

FIG. 2. Effects of DFP (○) and TOCP (△), which produced delayed paralytic syndrome, on the plasma and brain cholinesterases of the chicken. The results are expressed as percentage of the cholinesterase activity in control chickens killed on the same day as the poisoned chicken or chickens. One chicken in each group was sacrificed to obtain the data for the points in the curve for DFP. The average obtained from the enzyme activities of two chickens in each group was used to calculate the points in the curve for TOCP.

Although all the compounds tested inhibited brain true and/or pseudocholinesterase, the duration of the inhibition was different with each class of compounds tested, as is shown in Table 4 and Figs. 1 through 3. With the nonparalytic compounds, DDVP and Dipterex, a rapid recovery of inhibited brain cholinesterases took place (Fig. 1). However, in the case of the second group of organic phosphorus derivatives—those that produce paralysis after a delay of 10 to 14 days (Fig. 2)—it was found that with DFP a rapid recovery of the inhibited brain true or pseudocholinesterase occurred,

whereas with TOCP a prolonged depression of brain pseudocholinesterase was observed. These results with DFP and TOCP confirm previous work.[10, 11, 13–15] On the other hand, a prolonged depression of brain true and/or pseudocholinesterase was noted with each of the compounds (ronnel, malathion, EPN, and Trithion were

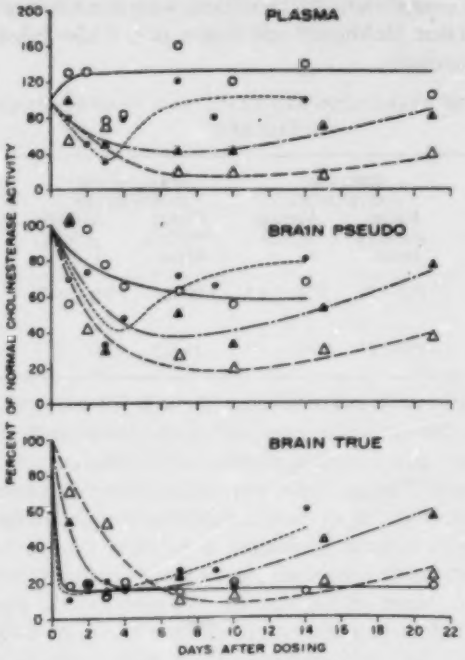

FIG. 3. Effect of malathion (●), EPN (○), Trithion (△), and ronnel (▲), which produce immediate paralytic condition, on plasma and brain cholinesterases of the chicken. The results are expressed as the percentage of the cholinesterase activity in a control chicken killed on the same day. Each point is calculated on the basis of one chicken in the control and experimental groups respectively.

tested) of the third group—those that produced paralysis without a prolonged delay.

The chickens given either EPN or Trithion refused food after dosing. Starvation has been shown to reduce the content of enzymes in the tissues under certain conditions.[34, 35] However, control experiments reported in Table 5, in which chickens were starved for as long as 12 days, show that there was no drop in the levels of the brain or plasma cholinesterases and that the animals did not develop characteristic muscle weakness. Therefore, the results obtained in the present experiment were not a result of starvation.

It also seems unlikely that the prolonged inhibition of cholinesterase found in the present experiments was an artifact caused by the reaction of previously separated enzyme and inhibitor during the process of homogenization of the brain. The most pronounced difference in levels of cholinesterase found between various compounds was obtained in those assays run 5 to 20 days after dosing, at which time any tissue level of inhibitor, if present, should be approaching a minimum. In addition, separate experiments in which malathion and DDVP were used as model compounds indicated

that homogenization artifacts did not occur. No inhibition of the brain cholinesterase in unpoisoned chickens was noted when homogenates of these brains were assayed in the presence of homogenates of the brains of chickens poinsoned 18 hr previously with malathion. Since maximal inhibition of brain cholinesterase was observed at this time with these two compounds, the conditions were most favorable for the presence of an excess of inhibitor. Hobbiger[26] and Seume *et al.*[27] also failed to obtain evidence of homogenization artifacts.

TABLE 5. EFFECTS OF STARVATION ON BRAIN AND PLASMA CHOLINESTERASES OF THE CHICKEN

Condition	No. of chickens	Brain true cholinesterase		Brain pseudo-cholinesterase		Plasma cholinesterase	
		Range μmoles/g brain	Average ± S.D.	Range μmoles/g brain	Average ± S.D.	Range μmoles 0·3 ml	Average ± S.D.
Starvation 8 to 12 days	3	163–176	174 ± 13	20–21	21 ± 0·71	6·4–9·9	8·5 ± 1·8
Normal	4	153–173	161 ± 9	19–23	21 ± 2·1	5·5–10·0	7·3 ± 2·0

No causative relationship exists between the delayed paralytic syndrome and the depression of brain cholinesterase since one of the compounds, DFP, that produces this syndrome did not cause a prolonged depression of this enzyme. On the other hand, a prolonged depression of brain true or pseudocholinesterase appears to be associated in some way with the syndrome of muscle weakness brought about by the compounds that produce paralysis without prolonged delay after dosing. In this case also, a cause-and-effect relationship apparently does not exist between the paralytic syndrome and the inhibition of brain cholinesterases. For example, the chickens sacrificed on the 15th or 21st day after the administration of ronnel, or the 14th day after administration of malathion, had recovered from the muscle weakness after 8 and 6 days, respectively, and yet the activity of brain cholinesterase was only 40 per cent of normal.

The results obtained in these studies with a variety of organic phosphorus compounds are in agreement with, and extend to other compounds, the conclusion reached in earlier studies with DFP, TOCP, and mipafox,[15, 16] that there is no causative relationship between the depression of the activities of brain cholinesterases and paralysis.

Vandekar and Heath[28] have shown that differences in rates of reactivation of cholinesterase can arise *in vitro* between compounds that form the same dialkyl phosphorylated enzyme if one compound persists longer than the other in the body. Their studies, together with those of Blaber and Creasey,[29] have shown that the rate of regeneration of cholinesterase *in vivo* depends on several competing reactions. The two that promote reactivation are: (1) spontaneous reactivation of the enzyme by hydrolysis and (2) synthesis *de novo* of new cholinesterase molecules. The two that oppose reactivation are: (1) persistence of the inhibitor in the body, causing re-inhibition of any reactivated enzyme, and (2) conversion of the inhibited form of the enzyme to a type that cannot be reactivated spontaneously or with oximes.

The comparatively rapid recovery of brain cholinesterase in chickens given DFP, DDVP, or Dipterex can be explained on the basis of the above theories. First of all, a linear plot of the logarithm of per cent inhibition of brain true cholinesterase,

calculated from data in Figs. 1 and 2 against time after dosing, showed that the half-time for the regeneration of this cholinesterase was 6 to 7 days with DDVP, Dipterex, or DFP. A similar plot for brain pseudocholinesterase revealed that the half-time of regeneration of this cholinesterase is 13 to 15 days for the same three organic phosphorus compounds. These half-times for the regeneration of brain cholinesterases are of the same order of magnitude as those calculated for the overall synthesis of brain proteins in other species.[30–33] Furthermore, the rate of synthesis varies with the cytological localization of individual protein and probably with the individual protein in the brain.[32, 33] Also, the regeneration of brain cholinesterases after inhibition with DFP *in vivo* has been considered the result of synthesis of new protein.[29, 34] Secondly, the ability of true cholinesterase in brain homogenates to be reactivated with 2-PAM, which is considered a measure of the reactivatable enzyme,[35–37] was completely gone 18 hr after dosing with DDVP.[41] Dipterex should form the same dimethyl phosphorylated enzyme as DDVP since both are dimethyl compounds. Therefore, the inhibited enzyme probably was in the irreversibly inhibited form before appreciable recovery of activity began in the brains of chickens given Dipterex. The same thing undoubtedly is true of the chickens given DFP since the half-time for the formation of the irreversible form of the diisopropyl phosphorylated cholinesterase is 4 hr.[26] These results suggest that at the dosages of DFP, Dipterex, or DDVP administered, brain cholinesterase was kept from reactivating by continued reinhibition with active inhibitor until all the inhibited enzyme had been converted to the unreactivatable form. Then regeneration of cholinesterase activity occurred by synthesis of new enzyme. It seems probable that what little spontaneous reactivation may have taken place was complete before the first test was made 1 day after dosing.

The rates of regeneration of brain true cholinesterase following dosing with those compounds that produced a prolonged inhibition of brain cholinesterase are more difficult to explain on the basis of the theories of Vandekar and Heath[28] and Blaber and Creasey[29] since it is necessary to indicate why protein synthesis did not regenerate the enzyme. In the case of the dimethyl compound, malathion, studies of the reactivation of brain homogenates with 2-PAM[41] showed that the enzyme was in the irreversible form 18 hr after dosing. Hobbiger[26] has shown that the half-time *in vivo* for the formation of the irreversible form of the diethyl phosphorylated brain cholinesterase is 36 hr. This is the alkyl phosphorylated enzyme that would be produced by the other compounds that cause prolonged inhibition of brain cholinesterase. Under these circumstances the enzyme regeneration must occur by protein synthesis.

The rate at which the plasma cholinesterase was depressed indicates that the compounds or their active derivatives that caused prolonged depression of brain cholinesterase were present in the body of the chicken longer than those that did not. Thus, as shown in Figs. 1, 2, and 3, a minimal level of plasma cholinesterase was reached one day after dosing with DDVP or Dipterex, whereas the time required to reach the minimal level ranged from 3 days with malathion to 10 days with Trithion. In all cases except that of the chickens dosed with TOCP, a slow recovery of the activities of brain cholinesterases began shortly after the time that the plasma cholinesterase activity had reached a minimum. No correlation of the rate of depression of plasma and brain cholinesterases could be made with EPN since the plasma enzyme was not depressed in chickens given this compound. These data are in accord with the concepts of Vandekar and Heath[28] and Blaber and Creasey,[29] and indicate that the brain

cholinesterase in the chickens given malathion, Trithion, TOCP, or ronnel may have been kept in the inhibited state by continued titration of any newly synthesized enzyme. On the other hand, it seems unlikely that this can be the complete explanation, particularly for the chickens given EPN or TOCP, since virtually no recovery of brain cholinesterase was observed. Repression of enzyme synthesis by substrate or related compounds has been demonstrated for several enzymes that metabolize drugs.[38–40] As a working hypothesis it is suggested that repression of the mechanisms for the synthesis of new cholinesterase molecules may also have contributed to the prolonged inhibition of brain cholinesterase noted with these compounds. However, no direct experimental evidence is available for this hypothesis.

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Exposure to Parathion

Effect on General Population and Asthmatics

Robert S. Ganelin, MD, Phoenix, Ariz, Cipriano Cueto, Jr., MS, Atlanta, and G. Allen Mail, MS, Tucson, Ariz

There has been much lay and medical concern regarding toxic effects of insecticides on the general population. In addition to direct toxic effects, it is suspected that insecticidal application has deleterious physical effects on persons with respiratory diseases. To evaluate some of these problems, the authors have attempted to quantitate actual absorption, symptoms, and laboratory evidence of intoxication in persons with varying degrees of exposure to parathion. By means of controlled observations, the respiratory effect of application of this compound on persons with bronchial asthma was also measured. The results suggest that the effect of this chemical on the general population is negligible.

THE ORGANIC phosphorus insecticides have become firmly established in agricultural use. Although the high toxicity of some of them to man is well known, it is recognized that proper safety precautions may obviate the dangers to persons with heavy occupational exposure.[1-3] However, concomitantly with the rapidly increasing use of insecticides, concern has been expressed by public health officials, insecticide appliers, farmers, and members of the general population about the hazard of poisoning to unprotected persons receiving small or incidental exposure. This has been of particular concern where residential areas are in juxtaposition to fields under cultivation.

Between 1951 and 1953, studies in Wenatchee, the apple-growing district of Washington,[4,5] led to the conclusions that depression of cholinesterase activity and symptoms of organic phosphorus poisoning occurred only after definitive exposure, and that persons without occupational or gross accidental exposure to insecticides were not affected by them. Although those conclusions were considered valid, a similar study was instituted in the Phoenix, Ariz area because of numerous complaints of poisoning from the general population and because of the differences between the Phoenix and Wenatchee areas which could conceivably lead to different results. These differences were (1) the preponderance of aerial rather than ground application of insecticides in the Phoenix area; (2) the large number of persons with respiratory, allergic, and other chronic diseases in the Phoenix area; and (3) differences in temperature, relative humidity, and other environmental factors.

Agricultural insecticides are used in the Phoenix area during most of the year. However, the heaviest applications are made to the cotton crop, especially in July, August, and September. Applications during June and October are smaller and the amounts of insecticides used for cotton in May, November, and December are almost negligible.

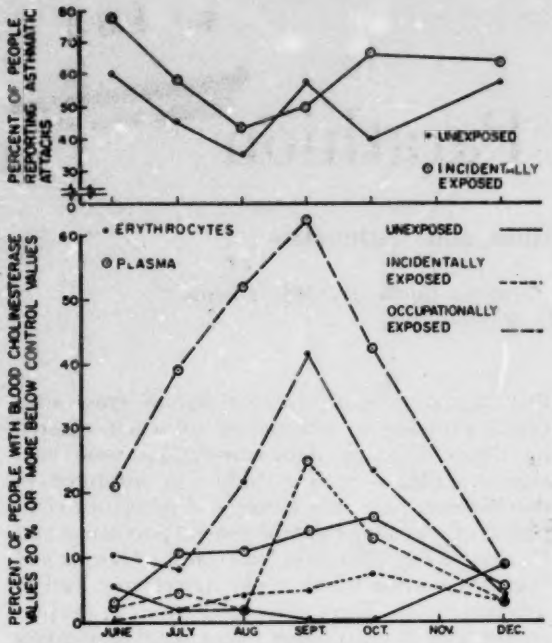
Methods

One hundred twenty-two volunteer subjects were placed in approximately equal groups in the following manner:

Group No.	Description of Group
1	Nonasthmatic, no exposure to organophosphorus insecticides
2	Nonasthmatic, environmental exposure to organophosphorus insecticides
3	Asthmatic, no exposure to organophosphorus insecticides
4	Asthmatic, environmental exposure to organophosphorus insecticides
5	Nonasthmatic, occupational exposure to insecticides, including organophosphorus compounds

Unexposed subjects lived and worked in the city of Phoenix and had no known contact with insecticides. Persons with environmental exposure lived less than 500 yards (less than 50 yards in the majority of cases) from cotton fields being treated by aerial application of insecticides. Asthma refers to the allergic type with unequivocal wheezing. The occupationally exposed group was composed of

From the Toxicology Section, Technology Branch, Communicable Disease Center of the Public Health Service, US Department of Health, Education and Welfare, and Maricopa County General Hospital, Phoenix (Dr. Ganelin); Department of Microbiology and Medical Technology, University of Arizona, Tucson (Mr. Mail); and the Toxicology Section, Technology Branch, Communicable Disease Center, Atlanta (Mr. Cueto).



Incidence of asthma and blood cholinesterase depression in people with different degrees of exposure to organophosphorus insecticides.

formulators, crop-dusters, loaders, and mechanics.

It is recognized, of course, that the entire population has some dietary exposure to insecticides.[6] However, in contrast to the chlorinated hydrocarbons, the active organophosphorus compounds, which hydrolyze very rapidly, are rarely found in detectable amounts on marketed crops. Thus it is felt that dietary exposure to organophosphorus compounds is negligible, and that groups 1 and 3 are truly "no exposure" groups.

In order to obtain pre- and postexposure observations, as well as data during the time of exposure, study members were visited monthly (except in November) from May through December, 1958. At each interview, blood and overnight urine samples were collected and the subject was questioned about insecticidal exposure and symptomatology. Symptoms investigated were asthma (as indicated by wheezing) and also disorders commonly associated with organic phosphate poisoning, namely, headache, blurring of vision, excessive lacrimation, nausea, weakness or fatigue, tightness in the chest without wheezing, insomnia, diaphoresis, fainting, and convulsions.

Table 1.—Reports of Any Combination of Three Symptoms* During Month Preceding Interview

	Groups 1 and 3 (Unexposed)		Groups 2 and 4 (Environmentally Exposed)		
Month	No. of Persons Interviewed	No. With Three Symptoms	Month	No. of Persons Interviewed	No. With Three Symptoms
June	34	3	June	40	1
July	46	2	July	43	3
Aug	45	4	Aug	42	6
Sept	42	0	Sept	40	4
Oct	43	0	Oct	38	3
Dec	35	1	Dec	33	2

*Headache, blurring of vision, excessive lacrimation, nausea, weakness or fatigue, tightness in chest without wheezing, insomnia, disphoresis, fainting, and convulsions (asthma excluded).

Cholinesterase activity of red blood cells and plasma was measured by a standard microelectrometric technique[7,8] following separation of red blood cells and plasma by centrifugation at 10,000 rpm for three minutes. Urinary p-nitrophenol was measured by the method of Elliott et al[9] and was expressed as a parathion equivalent.

Results

Symptoms of Organophosphorus Poisoning.—Table 1 presents the number of persons with no exposure or with environmental exposure who reported any combination of three symptoms (excluding asthma) for the month preceding each interview. Although the number complaining of symptoms was higher for the exposed groups, the differences are not significant for any month. No person reporting three symptoms appeared clinically to have organophosphorus poisoning; only three had depressions of cholinesterase activity of red cell and plasma of 20% or more; none had depression of both red cell and plasma cholinesterase activity concomitantly with his symptoms. There was no report of three symptoms from the occupationally exposed group (between 19 and 23 were interviewed each month), although a few gave histories of miosis and headache which were related to exposure.

Cholinesterase Depression.—The percentage of persons with a decrease of 20% or more from their own pre-exposure cholinesterase activities is shown by month in the lower portion of the Figure. As no significant differences were found between asthmatics and nonasthmatics in either the exposed or unexposed groups, the data are combined according to exposure. The differences between unexposed and incidentally exposed persons were small and not significant ($P<0.05$), while the differences between the occupationally exposed group and the other two groups combined were significant for plasma in June and July and for both plasma and red cells in August, September, and October.

Excretion of p-Nitrophenol.—Of the 320 urine samples collected from persons in groups 1, 2, 3, and 4, only one contained material which gave a reaction for p-nitrophenol. This positive specimen was from a farmer in group 4 who was excreting 8μg per hour. He had no known exposure during the previous month, no symptoms of poisoning, and no decrease of cholinesterase activity of the blood.

Table 2.—Rate of Excretion of Urinary p-Nitrophenol* by Occupationally Exposed Persons

No. of Persons	May, μg/hr	June, μg/hr	July, μg/hr	Aug, μg/hr	Sept, μg/hr	Oct, μg/hr	Dec, μg/hr
Pilots							
2	0†	12‡	6	71	31	0	0
3	....	....	47	65	0	45	0
5	0	....	3	29	0	8	0
7	0	....	0	16	10	0	0
10	0	0	0	4	4	....	0
13	....	0	....	106	....	0	0
14	0	....	6	86	10	18	0
15	trace	....	94	53	259	....	2
18	0	....	....	118	8	0	0
19	0	....	0	....	....	....	0
24	0	....	4	12	37	0	0
25	0	....	10	35	4	0	0
26	....	....	46	14	6	0	....
Loaders and Mechanics							
4	0	....	....	80	73	22	0
8	trace	....	..	....	14	0	1
9	0	0	16	0	8	0	0
12	trace	....	24	68	392	35	....
16	0	....	14	33	24	12	2
20	0	....	0	....	....	0	0
Formulators							
17	0	....	0	8	4	6	0
21	....	....	0	12	4	24	0
23	0	....	12	145	6	86	18

*Expressed as parathion equivalent in μg/hr.
†Value of zero means that any quantity present was below sensitivity of method.
‡The rates have been "rounded" to the nearest whole number.

The data in Table 2, however, show that the persons in the occupationally exposed group frequently excreted p-nitrophenol, particularly during the peak periods of July, August, and September. During August, only one person, a formulator, did not excrete measurable p-nitrophenol.

Symptoms of Asthma.—The upper portion of the Figure shows the percentage of persons in groups 3 and 4 who complained of asthma at any time during the month preceding interview. The amount of asthma was remarkably similar in the two groups except in October, a month of comparatively little insecticidal exposure for group 4. A somewhat higher proportion of persons with incidental exposure reported asthma except during September, the month when the effects of exposure as measured by cholinesterase were greatest in men with occupational exposure.

Comment

The results of this study support the conclusions of others [5,7,10,11] that hazards of insecticidal poisoning to persons with environmental or incidental exposure are negligible. The environmentally exposed persons in this study probably had as much exposure as any individuals not occupationally involved with insecticides. Most of their homes were directly across a road from the fields being treated and were frequently enveloped by drift following application. Although the occupants, whether asthmatic or not, frequently complained about the insecticides, primarily because of odor and irritation of mucous membranes, there was no individual who had symptoms resembling those of organophosphorus poisoning.

In addition to the lack of symptoms of poisoning, two other factors indicated that hazard of environmental exposure was negligible. First, there was not a significant incidence of cholinesterase depression in groups 2 or 4 when compared with their controls. A 20% decrease was chosen because it has been recognized that variation between measurements on different samples from the same unexposed person may be as high as 23% for red blood cells and 25% or more for plasma.[12,13] That depressions of 20% are not necessarily significant of poisoning is indicated by the fact that there were as many such depressions in unexposed persons as in those with some exposure. Lack of any cholinesterase depression in these subjects when they had symptoms is, however, corroboration of the clinical impression that the few symptoms reported were not related to insecticides.

Second, measurement of the p-nitrophenol excretion indicates that the actual insecticidal exposure of all persons other than those in the occupational group was extremely small. Since parathion was the organophosphorus insecticide used almost exclusively in the area under study, information concerning it or its measurable metabolite should be representative of exposure to all insecticides used. We have found that excretion of p-nitrophenol during the first 24 hours after exposure appears to be a more sensitive index of exposure than either symptoms or cholinesterase activity; many of the specimens from groups 2 and 4 were collected within that interval. In persons with occupational exposure, significant amounts of this metabolite were found almost uniformly.

Evaluation of the effect of insecticides on asthma is much more difficult than evaluation of its effect as a poison. The asthmatics with environmental exposure were generally older than their controls, had asthma longer, and had a higher incidence of chronic respiratory disease, such as pulmonary emphysema and bronchiectasis. These factors would tend to make the incidence of asthma higher in the exposed group. Despite this, the incidence in the two groups was remarkably similar and roughly followed the same seasonal occurrence. This similarity suggests that the morbidity may have been due to common factors such as molds, grasses, and weeds in June, and house dusts and bacterial antigens in the winter months.

However, it is difficult to disregard the fact that many of the exposed asthmatics unequivocally indicated temporal relationships between exposure to insecticides and asthmatic attacks. One defect in evaluating the data is that no attempt at quantitating the asthmatic attacks was made. This, plus the complexity and the variability of the disease, makes it impossible to draw definitive conclusions regarding the relationship between environmental

exposure to insecticides and exacerbation of asthma. However, these similarities in occurrence do indicate, despite the drawbacks of the method, that if insecticides do affect pre-existing bronchial asthma, the effect is a relatively minor one.

4402 N Seventh St, Phoenix, Ariz 85014 (Dr. Ganelin).

Dr. Wayland J. Hayes, Jr. suggested that this study be undertaken and Dr. Melvin H. Goodwin, Jr. aided in the completion of the project and the preparation of this manuscript.

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Printed in U.S.A.

PB 280 569

PUBLICATION

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TOXICOLOGY PROGRAM
Technology Branch
Communicable Disease Center
Atlanta 22, Ga.

Reprinted From The Journal of The American Medical Association
November 24, 1962 Vol. 182, pp. 848-850
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Potentiation of Phosphorus Insecticides by Phenothiazine Derivatives

Possible Hazard, with Report of a Fatal Case

John D. Arterberry, MD, Robert W. Bonifaci, MD, Edward W. Nash, MD, and Griffith E. Quinby, MD, Wenatchee, Wash.

A mentally deficient apple rancher developed moderate signs of organic phosphorus poisoning when his blood cholinesterases were inactivated to near zero after exposure to Phosdrin and parathion. Symptomatic therapy included promazine on the 9th through the 12th days of illness. The patient became more agitated, and some signs of poisoning returned during attempted tranquilization. After about the 8th dose of promazine (Sparine) hydrochloride in 3 days, the patient developed convulsions, hypotension, and apnea, and died. The possible hazard of phenothiazine-derived drugs may outweigh their therapeutic value in the treatment of organic phosphorus poisoning.

SINCE 1952, French,[1] Swedish,[2] and American [3] workers have demonstrated prophylactic or therapeutic benefits of phenothiazine derivatives in experimental poisoning by organic phosphorus compounds.

Chlorpromazine hydrochloride with atropine (sometimes with adrenocorticotrophic hormone) has been advocated in Italy,[4] but not, to our knowledge, in the United States, in the treatment of people poisoned by organic phosphorus compounds. However, many cases of poisoning have been treated with various phenothiazine-derived drugs in this country, principally because of their antiemetic and tranquilizing effects. Neither adverse nor notably beneficial effects were reported from approximately 50 such treated cases mostly from California.[5]

From the Toxicology Section, Technology Branch, Communicable Disease Center, Public Health Service, U.S. Department of Health, Education and Welfare (Drs. Arterberry and Quinby). Dr. Arterberry is now with the Department of Pharmacology, University of Southern California, School of Medicine, Los Angeles.

The truly potentiating effects of the phenothiazine-derived compounds are not to be confused with simple additive effects or other severe and diverse side effects. Synergism has been reported [6] between phenothiazine itself and 2 organic phosphorus esters used to de-worm sheep.

There are numerous reports of inhibition of plasma cholinesterase by phenothiazine derivatives in vitro in rats, horses, and man, but only 2 investigators have recorded the same effect in vivo in man [7] and in dogs.[8] Most investigators believe that the pharmacologic action of phenothiazine derivatives cannot be explained in terms of their anticholinesterase effect, even though such an effect is recognized. To our knowledge, the anticholinesterase effect of phenothiazine derivatives has never been established as clinically important in man. However, 2 Danish pharmacologists [9] have recently demonstrated complete inhibition of human brain cholinesterase in vitro by chlorpromazine using a different assay method.

Contraindications to the use of phenothiazine-derived drugs, whether based on potentiation or on side effects, have not heretofore been specifically directed toward use in treating patients poisoned with organic phosphorus compounds. The delayed, unexplained death of an orchard-worker, with near-total cholinesterase inactivation after exposure to insecticides, led us to suspect potentiation with promazine as the cause of death. Instead of being of benefit in treatment of Phosdrin poisoning, as suggested by Italian investigators,[4] Dr. Mitchell Zavon of the Kettering Laboratory at Cincinnati, found that chlorpromazine increased the mortality rate in Phosdrin-poisoned rats both when given alone and when combined with atropine. Later investigations in our own laboratories showed that potentiation can occur in rats between promazine or chlorpromazine and parathion. It was also demonstrated between promazine and parathion in guinea pigs (Gaines, Thomas B., manuscript to be

published). The present case report resembles those reported by others who suspected synergism between phenothiazine relatives and other neurotropic drugs such as anesthetics, antidepressants, antihistamines, and tranquilizers with mixed pharmacologic actions.

Report of a Case

A 47-year-old mentally deficient orchard worker developed an "upset stomach" on June 8, 1959, sometime during or after spraying Phosdrin. He wore an approved respirator and protective rubber clothing at least part of the time. He applied 5 tanks (400 gal. each) of Phosdrin from morning until noon with a speed-type sprayer. In the afternoon he applied Sevin. However, this carbamate insecticide has such a low toxicity, both as a cholinesterase inhibitor and as a muscle blocking agent, that it is not further considered in this paper.

The patient went into town probably without having bathed. He was brought home early that night complaining of nausea, and was perhaps more than usually mentally confused. He remained ill all night. By the next day he was oriented as well as usual, but had a cough and moderate respiratory difficulty. Because of the respiratory signs and delay of the full-blown illness until 9 or 10 hours after last exposure to an organic phosphorus compound, prevalent epidemic gastroenteritis was first suspected, rather than poisoning.

First symptomatic treatment included morphine one-sixth grain and atropine sulphate 1/150 grain. The patient felt much better the following day (June 10), and he sneaked out of bed and returned to more spray work. He applied parathion, but he never revealed details. The reexposure made him more ill.

On the fourth day of illness, the patient was weaker and ataxic. Miosis was first noted then. The physician returned his attention to the possibility of organic phosphorus poisoning. Cholinesterase activity of the red blood cells was 0.02 and that of plasma was 0.00 Δ pH per hour by the method of Michel.[10]

Atropine tannate (1 mg.) by the oral route, was prescribed 4 times a day for 5 days. The patient was seen at home by the attending physician and toxicologist several times between the 5th and 11th days of illness. This included 2 visits on the 9th day, at which time restlessness was of first and major concern. Accordingly, 50 mg. of promazine was given intramuscularly, and an additional supply of oral tablets was left with his parents. It was estimated that 6 oral doses of promazine were given from the 9th through the 11th days before he entered the hospital. He wandered about the house at night, weak, confused, and restless.

Upon admission to the hospital at 9:30 P.M. of the 11th day of illness, the patient was coughing, in respiratory difficulty, slightly cyanotic, and miotic. His temperature was 101° F. (38.33° C.), his blood pressure was 190 systolic and 120 diastolic, and his pulse was 108. The white blood cell count was 21,800.

Shortly after admission, 50 mg. more of promazine was given intramuscularly. He appeared to rest better for several hours. By midnight, however, he had a profuse diaphoresis. He insisted on sitting on the side of his bed. He dozed at intervals during the rest of the night. Early during the second hospital day he became more irrational, he convulsed lightly, and he became incontinent 3 times in 1 hour. A second hospital dose of promazine (50 mg. intramuscularly) was given at 6:00 A.M. Five minutes later his blood pressure had declined to 130 systolic and 90 diastolic. His pulse and color rapidly became so poor, that he was believed to have experienced vascular collapse. Fifteen minutes later, respiratory difficulty increased and cyanosis deepened. He was placed in an oxygen tent, and his first hospital dose of atropine sulphate (1/150 grain) was given, and was repeated 25 minutes later. The pulse was a little stronger for a short time. Blood drawn at 6:30 A.M. later revealed that red cell cholinesterase activities had not significantly increased (0.05 Δ pH per hour) during the 9 days since last exposure, but plasma cholinesterase activity had risen to 0.44 Δ pH per hour.

The vascular collapse was treated at 7:00 A.M. with an infusion of 1,000 cc. of dextrose in saline containing 4 cc. or 2 mg. of levarterenol (Levophed) bitartrate at 40 drops (4 mcg. of active drug) per minute.

At 8:15 A.M. a respirator with airway was used to support respiration, since the patient's skin color still indicated inadequate ventilation. By this time, blood pressure had decreased to 100 systolic and 60 diastolic. Although his color improved in the next 15 minutes because of mechanical ventilation, the blood pressure slipped further to 90 systolic and 50 diastolic.

By 8:40 A.M., in response to the circulatory stimulant, the pressure had risen to 130 systolic and 60 diastolic, and at 9:15 A.M., color remained good. Pulse and blood pressure were stronger for the next 2 hours.

By 11:15 A.M., respirations became shallow and the extremities were mottled. The patient's temperature had risen to 103.6° F. (39.8° C.) rectally, and later, at 12:30 P.M., a third dose of 1/75 grain atropine sulphate was given intramuscularly and a second 5 per cent intravenous dextrose infusion was started.

At 1:00 P.M., blood pressure was not obtainable, pulse was thready, and cyanosis was greater. A little later, the patient had no blood pressure or pulse. He ceased to breathe, and was pronounced dead at 1:20 P.M.

Autopsy Findings.—Analysis of postmortem blood indicated that plasma cholinesterase activities had risen still further (0.52 Δ pH per hour), but that of the red blood cells had not (0.08 Δ pH per hour). Postmortem spinal fluid had a cholinesterase activity within the low range of normal as recently determined in our laboratory by the Michel method.

The gross and microscopic autopsy results were summarized as follows: (1) left ventricular dilation, (2) slight coronary atherosclerosis, (3) pulmonary congestion and edema, (4) cerebral edema, (5) anterior horn cell degeneration consistent with the history of poliomyelitis, and (6) delayed restoration of red blood cell cholinesterase activity.

Comment

The patient's mental deficiency caused the clinical and exposure histories to be incomplete and possibly unreliable.

The earliest recognizable onset with an "upset stomach" occurred during, or at most, a few hours after applying Phosdrin. The patient began to recover, but became worse after exposure to parathion. Paranitrophenol, a product of the hydrolysis of parathion, was determined[11] in the urine. The concentrations were highest (0.90 p.p.m.) in the first specimens collected for analysis on the first day of exposure to parathion, and declined progressively to a level of 0.25 p.p.m. on the sixth day afterwards. The levels in the present case were a little above those of 2 heavily exposed individuals studied earlier.[12]

The practically complete inactivation of both blood cholinesterases the day after exposure to parathion, suggested that the additional exposure to the parathion was considerable. One does not

find such severe inactivation, especially of red blood cell cholinesterase, in unpoisoned periodic applicators, although very low levels of blood cholinesterase without symptoms have been found in formulators with prolonged exposure.[13] Therefore, all available evidence collected in the first few days of illness indicates he was poisoned by Phosdrin or parathion or both, before he was given promazine on the ninth day. Late in the course the symptoms seemed even more cholinergic in pattern. During the last few hours before death the patient had miosis not relieved by atropine, profuse diaphoresis, cyanosis, and muscle twitchings, in spite of rising plasma cholinesterase activity indicative of partial recovery from acute poisoning. These cholinergic signs and symptoms just before death support a diagnosis of death by cholinesterase inactivation; the rising plasma cholinesterase after survival for the protracted period since exposure contends against this impression as the sole cause of death, unless we accept the evidence that the Michel method has frequently failed to detect enzymes reversibly-inhibited by phenothiazine derivatives.[9]

The mechanism of action of potentiation of organic phosphorus compounds by phenothiazines has yet to be proved. The normal passage of nerve impulses depends both on effective levels of active cholinesterase and on the acetylcholine protein receptors on the interneuronal membrane.[14] Blockage of electrical activity by some amino neurotropic agents occurs only when acetyl cholinesterase activity is very low.[14] The first detection of synergism between amino neurotropic drugs (theophylline and theophylline-ethylenediamine) and an organic phosphorus compound (DFP) was reported by Johns et al.,[15] in 1951, in measuring the convulsive thresholds of bronchodilators and DFP in rabbits. These workers offered proof that the mechanism of the sixfold potentiation was some interaction in addition to the anticholinesterase effect. Their data also suggest that brain cholinesterase may have to be near zero before this synergism occurs. However, the more recent recognition of the reversibility of cholinesterases totally inhibited by chlorpromazine[9] may explain the "paradoxical" action of phenothiazine derivatives at high concentrations. It appears possible, therefore, that organic phosphorus poisoning may be benefited in both man[4] and experimental animals if phenothiazine-derived drugs are administered in the presence of active acetyl cholinesterase. However, when acetyl cholinesterases reach a sufficiently low level, both organic phosphorus anticholinesterases and neurotropic amine drugs, such as the phenothiazine derivatives, may act respectively on acetylcholine or its receptor protein to alter transmission of nerve impulses, and thus to produce potentiation. The promazine in our case may have partially, but reversibly, inhibited the small percentage of still-active cholinesterases remaining after poisoning by the 2 organic phosphorus compounds. In any event, the multiple administration of promazine from the 8th to the 12th day of only moderate illness after exposure to Phosdrin was followed by increased cholinergic signs, and ultimately by a severe fall in blood pressure, convulsions, respiratory failure, and death.

This case report should alert physicians to the fact that the possible hazard of phenothiazine-derived drugs may outweigh their therapeutic value in the treatment of organic phosphorus poisoning.

P.O. Box 73, Wenatchee, Wash. (Dr. Quinby).

Generic and Trade Names of Drugs

Promazine hydrochloride—*Sparine Hydrochloride.*
Levarterenol bitartrate—*Levophed Bitartrate.*

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PB 280 570

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Acta Dermato-Venereologica, Vol. 41, p. 353–362 (1961)

TOXICOLOGY PROGRAM
Technology Branch
Communicable Disease Center
Atlanta 22, Ga.

STUDIES ON THE PERCUTANEOUS ABSORPTION OF PARATHION AND PARAOXON

III. Rate of Absorption of Parathion

TORSTEN FREDRIKSSON*
ATLANTA, GEORGIA, U. S. A.

As has been stated in an earlier publication in this series, Fredriksson *et al.*, 1961, there is no detailed knowledge in experimental animals or man concerning the percutaneous absorption of parathion, one route of intoxication with this widely used organophosphorus insecticide. In particular, there is no information about its rate of absorption. The aim of the present study was, therefore, to try to measure the rate of percutaneous absorption of parathion in both animals and man. For obvious reasons, it is ideal to perform such experiments *in vivo*, *e. g.* determination of the disappearance rate from a topical deposit of radioactive material. In the case of three other more water soluble compounds, this method allowed measurements of rates less than 10 μg min^{-1} cm^{-2}, Fredriksson, 1958, and it is thus extremely sensitive. However, parathion could be expected to show a lower rate of absorption, approaching the sensitivity limit of the method, due to its different solubility properties. It has very low water solubility, Metcalf, 1955, and it is generally agreed that compounds with high oil/water-partition coefficients are less liable to absorption than substances with intermediate values, Hadgraft and Somers, 1956. It was, therefore, decided to do experiments *in vitro* also, using essentially the method of Treherne, 1956, that is even more sensitive than the method used *in vivo*, even though results so obtained probably are less reliable than data recorded *in vivo*.

Methods

^{32}P-labelled parathion (E 605, or diethyl 4-nitrophenyl thiononophosphate) with an initial specific activity of 1.75 mC per mM, furnished by Volk Radiochemical Company, Chicago, Illinois, was used in all the experiments. When tested for purity, Fredriksson *et al.*, 1961, it was found to contain less than 0.1 per cent of an unidentified contaminant.

Disappearance measurements were performed in essentially the same way as described earlier, Fredriksson, 1958. Three female cats, weighing between 3.2 and 3.8 kg, were used. The animals were anaesthetized with sodium pentobarbital (30 mg per kg body weight) administered intraperitoneally. However,

Received for publication May 31, 1961.
* Dr. Fredriksson was a Visiting Scientist. *Present address:* Department of Dermatology, Karolinska sjukhuset, Stockholm 60, Sweden.

Toxicology Section, Technology Branch, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia, U. S. A., and the Department of Dermatology, Karolinska sjukhuset, Stockholm 60, Sweden.

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due to the present laboratory equipment, more superficial anaesthesia had to be used, with the result that the distance between the deposit and the detector was less constant, and the disappearance curves were therefore somewhat distorted. Fifty μl of undiluted labelled parathion was applied on an area of 4.1 cm^2, demarcated with an aluminum ring fixed to the skin with collodion. A Nuclear-Chicago model D 34 thin mica window (1.4 mg per cm^2) GM-tube, operated at 950 volts, was used together with a Nuclear-Chicago recording rate meter model 1620 B; the time constant was set at 40 seconds. From the curves obtained, the disappearance constant k was calculated as in the earlier investigation. Blood samples were taken every 60 minutes during the five hours of observation for determination of cholinesterase activity in plasma, using acetylcholine as substrate and Warburg technique. Blood samples for determination of radioactivity were taken every 30 minutes, usually starting 2 hours from application. Two ml of heparinized blood were diluted to 8 ml with tap water (except the last sample in each experiment, which was 8 ml and could be used undiluted). A Nuclear-Chicago model D 52 GM-tube, having a wall thickness of 35 mg per cm^2 and operated at 1110 volts, was used as a dip counter, together with a Nuclear-Chicago model 162 binary scaler in these activity determinations. Counts were recorded over a 25-minute period, and background activity was determined before and after each series of determinations for the same period of time. For calibration of these values, labelled parathion was diluted with acetone; the activity in 8 ml samples of three different concentrations was determined with the same equipment.

The method described above was found to lack sufficient sensitivity to be used in human experiments, when the material was applied to intact skin. This was due to the fact that the distance between deposit and detector could not be kept as constant as necessary for estimation of the apparently low disappearance constant. However, in human skin where the barrier had been destroyed by 25 consecutive strippings with transparent cellulose tape, it was possible to get an estimate of the rate of absorption. Only three experiments on one individual were performed, since such studies seemed to have limited theoretical value. The same techniques used in the cat were followed in applying the compounds in this experiment, using a stripped area on the back of the hand. The activity was determined during 2-minute periods every 15 minutes for three hours, with equipment described elsewhere, Fredriksson, 1961.

In the experiments performed *in vitro*, a plexi-glass absorption chamber, shown in Fig. 1, was used. In clamping the skin in chambers of this type, there are always risks of injury to the barrier, where the skin is compressed by the upper plexi-glass ring, resulting in increased absorption. In order to reduce errors due to such leakage, a ring of stainless steel was fixed to the skin with collodion, which was allowed to dry for 30 minutes. The lower compartment was filled with 0.9 per cent saline or heparinized blood, gently stirred with the aid of a magnetic device. In these experiments, which were performed at 21—22° C, skin from rat (Sherman strain), cat, and man were used. The rats were killed by a blow on the head, and skin from the back, clipped close to the surface, was removed immediately after death. Subcutaneous tissues were carefully removed by blunt dissection, and the ring, which had an inner area of 3.1 cm^2, was attached to the epidermal side under gentle stretching of the skin. It was

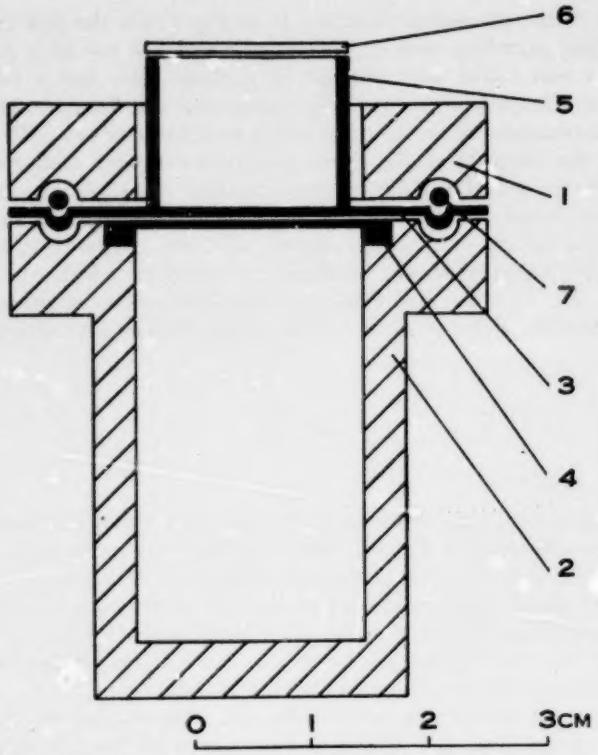

Fig. 1. Plexi-glass absorption chamber. 1, application portion; 2, receptor portion filled with saline or blood; 3, skin; 4, fine mesh screen grid used as support for skin; 5, metal ring glued to skin; 6, cover glass; 7, o-ring rubber gasket.

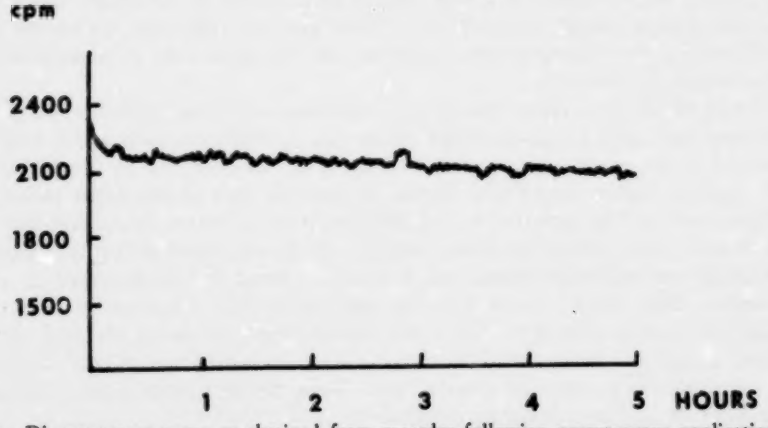

Fig. 2. Disappearance curve as obtained from recorder following percutaneous application of ^{32}P-labelled parathion. Cat No. 2, 3.2 kg, sodium pentobarbital. 50 μl parathion applied on an area of 4.1 cm^2.

then placed in the plexi-glass chamber, as in Fig. 1. On the skin surface, 50 μl of the labelled parathion was applied evenly with the aid of a glass rod, and the chamber was sealed with the aid of a cover glass and a lubricant. The chamber was clamped on a rigid ring-stand, and the disappearance of radioactivity was measured as in the experiments performed *in vivo*. Five hours after application the contents of the lower compartment were analyzed for radioactivity with the aid of the dip counter described above. In the cat, skin from the back was removed under sodium pentobarbital anaesthesia, but was otherwise treated as in the rat. Human sternal skin was obtained at autopsy, performed within 10 hours of death. These skin samples, which were from adult individuals of both sexes, had evidently not been washed or otherwise treated and showed no evidence of disease. The human skin was treated as in the rat and the cat.

Results

Experiments in vivo

The disappearance measurements in the cat, which are illustrated by Figs. 2 and 3, and summarized in Table I, gave essentially the same result in the three animals. No local or systemic cholinergic symptoms were noted. On the inside of the cover glass, which was located on the metal ring, colorless droplets appeared approximately 30 minutes after application. When analyzed after the experiments, this liquid was found to contain no ^{32}P-labelled material, indicating that no measurable evaporation had occurred.

The failure to completely immobilize the animals led to distorted disappearance curves (Fig. 2). This, in conjunction with the low rate of absorption, made it impossible to calculate the disappearance constant with the same degree of accuracy as had been accomplished earlier with sarin and its analogues, Fredriksson, 1958. However, when counting rates, arbitrarily taken from the curves, were plotted in a semi-logarithmic system as ordinates against the time, an approximately straight-line relationship was obtained. From the slope of this curve, the disappearance constant and the mean rate of absorption was then estimated (Table I).

In two of the cats, there was a slight tendency towards inhibition of plasma cholinesterase activity, as exemplified in Fig. 3, while no such trend could be observed in the third experimental animal. The radioactivity in the blood was only slightly higher than the background until the end of the experiments, but as illustrated in Fig. 3, there was a definite trend towards increasing activity. The second count from the final sample, which was more accurate because it was made on undiluted blood, was more than twice the background in all of the cases. (The second count does not appear in Fig. 3 because it was made under different conditions.) The final radioactivity, as obtained from the undiluted samples, corresponded to 0.02 to 0.04 μg parathion per ml of blood. This radioactivity may, of course, have been derived from both unchanged parathion or its ^{32}P-containing metabolites.

The three disappearance curves obtained from stripped human skin *in vivo*, as exemplified in Fig. 4, had the same general appearance and approximately the

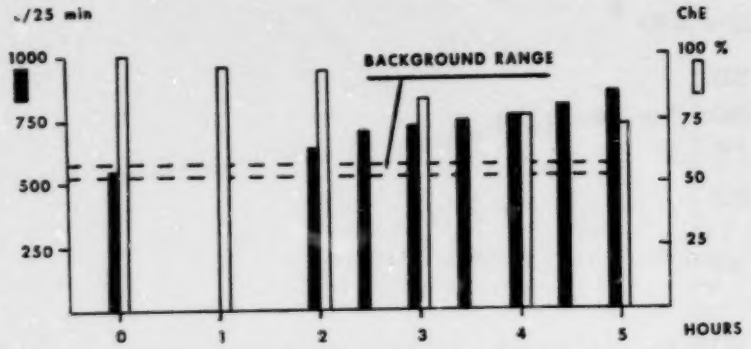

Fig. 3. Radioactivity in blood and plasma cholinesterase activity following percutaneous application of ^{32}P-labelled parathion in cat No. 3.

Table I. *Disappearance constants and mean rates of percutaneous absorption as obtained in vivo after administration of 50 µl ^{32}P-labelled parathion on 4.1 cm^2 in the cat.*

Cat No.	Disappearance constant min^{-1}	Mean rate of absorption	
		µg min^{-1} cm^{-2}	mµ M min^{-1} cm^{-2}
1	9×10^{-5}	1.4	5
2	10×10^{-5}	1.6	6
3	8×10^{-5}	1.2	4

same slope. The disappearance constant varied between 0.0008 and 0.0014, which corresponds to a mean rate of absorption of 12—21 µg min^{-1} cm^{-2}, or 40—70 mµ M min^{-1} cm^{-2}. In these experiments also, there were no local or systemic symptoms.

Experiments in vitro

The experiments performed with the absorption chamber are illustrated by Fig. 5, and summarized in Table II and III. As can be seen in Fig. 5, there was no measurable decrease in radioactivity in these disappearance experiments, mainly due to incomplete self-absorption of β-rays. Table II also shows that in all the species tested, the rate of absorption, as based on the radioactivity found in the recipient portion of the chamber at the end of the experiments, was much slower than the rate found in the cat *in vivo*. When blood was used instead of saline in the recipient, there was a tendency towards increased measured activity (Table III).

Comment

The values given in Table I, regarding the rate of percutaneous absorption of parathion *in vivo*, are most uncertain for reasons previously stressed. It is, however, evident that parathion is much more slowly absorbed in the cat

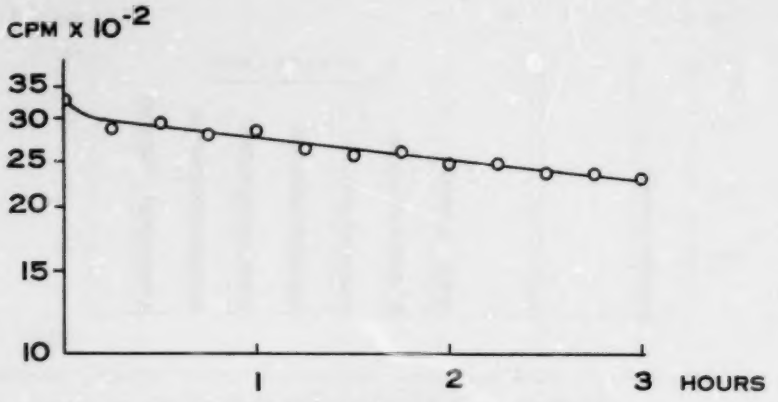

Fig. 4. Disappearance curve following application of ^{32}P-labelled parathion on stripped skin in man.

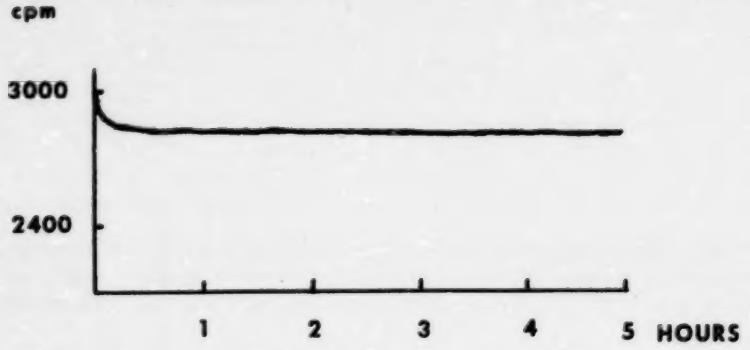

Fig. 5. Disappearance curve as obtained from recorder *in vitro* following application of ^{32}P-labelled parathion on excised cat skin.

Table II. *Mean rate of absorption of parathion in three species as obtained in vitro with saline in recipient portion of chamber.*

Species	Experiment No.	Mean rate of absorption	
		$\mu g\ min^{-1}\ cm^{-2}$	$m\mu\ M\ min^{-1}\ cm^{-2}$
Man	1	8×10^{-4}	27×10^{-4}
	2	10	34
	3	10	34
Cat	1	18	62
	2	21	72
	3	17	59
Rat	1	14	48
	2	11	37
	3	16	55

Table III. *Mean rate of absorption in the cat as obtained in vitro with heparinized cat's blood in recipient portion of chamber.*

Experiment No.	Mean rate of absorption	
	$\mu g\ min^{-1}\ cm^{-2}$	$m\mu\ M\ min^{-1}\ cm^{-2}$
1	48×10^{-4}	165×10^{-4}
2	59	200
3	42	144

than is sarin. The rate of absorption of this compound under similar experimental conditions was found to be 18 $\mu g\ min^{-1}\ cm^{-2}$, corresponding to 129 $m\mu\ M\ min^{-1}\ cm^{-2}$, Fredriksson, 1958. When compared on a molar basis, sarin is thus absorbed around 25 times faster than parathion. This is what can be expected from the solubility properties of these compounds, Hadgraft and Somers, 1956.

This slow rate of absorption of parathion is supported also by the fact that less radioactivity was demonstrated in blood than with sarin. However, the amount of radioactivity in blood is dependent upon the actual distribution of the labelled material. Another investigation, Fredriksson and Bigelow, 1961, demonstrated that there was a very low level of radioactivity in blood, as compared with many other tissues, following subcutaneous injection of labelled material in the mouse.

There was only noticeable inhibition of plasma cholinesterase activity in two of the animals, and no cholinergic symptoms were observed. Parathion thus shows a much lower percutaneous toxicity than sarin, and this, of course, is mainly due to its lower systemic toxicity. However, the lower rate of absorption of parathion may result in a relative decrease in its percutaneous toxicity, since slow systemic absorption in general may involve more effective detoxification, Holmstedt, 1959.

The slow rate of absorption of parathion makes this compound most unsuitable as a model substance in studies of the process of percutaneous absorption. Thus, it was not possible to determine the rate of absorption *in vivo* through intact skin in man. Such data are not only of theoretical interest but also have practical importance within the field of occupational dermatology. The reason is that such values would give a more proper foundation for extrapolations from experimental animals to man in the assessment of dermal toxicity due to possible species variation in rate of absorption. The results obtained *in vitro* do not indicate any great species differences. However, these values do not have full bearing on conditions *in vivo* and may reflect instead the capacity of the corium to retain the absorbed material. Even if the concentration of the labelled material in the recipient was much lower than the solubility limit of parathion in water, Metcalf, 1955, the unfavorable distribution factor might decrease the release of the material from the subepidermal tissues, possibly to a different extent in the various species. This explanation is supported by the fact that a somewhat greater activity was demonstrated in the recipient part of the chamber, when blood was used instead of saline.

The fact that a measurable rate of absorption could be determined in the

case of stripped human skin, but not in intact skin, illustrates the well known fact that injuries to the epidermal barrier will increase the rate of absorption, *e. g.* Griesemer, 1959. This has definite practical implications, since injuries to the skin surface are bound to occur under actual occupational conditions. This factor, absorption from large skin areas, and the risks of continuous absorption, Fredriksson, 1961, may explain the occasional occurrence of percutaneous intoxications among spray operators, workers in formulation plants, and others, even though the rate of absorption through intact skin has been shown to be very low in man.

SUMMARY

The rate of percutaneous absorption of parathion (E 605, or diethyl 4-nitrophenyl thiononophosphate) has been estimated *in vivo* by following the disappearance from a topical deposit of ^{32}P-labelled material (50 μl applied on 4.1 cm^2) in the cat. The rate of absorption was very slow, approximately 5 mμ M min^{-1} cm^{-2}, which is close to the sensitivity limit of the method and about one twenty-fifth that of sarin. There was a definite tendency towards inhibition of plasma cholinesterase activity in two cats, while a third animal failed to show this. The amount of radioactivity in the blood five hours after the application corresponded to 0.02 to 0.04 μg parathion per ml.

The disappearance technique was found to lack sufficient sensitivity to determine the rate of percutaneous absorption in man, except when the epidermal barrier was injured with adhesive tape.

The rate of absorption was also studied *in vitro* with excised skin from man, cat, and rat, by determining the amount of radioactivity in the recipient portion of an absorption chamber. The rate of absorption through the excised skin was much slower than the systemic absorption *in vivo* in the cat, and it was concluded that the *in vitro* method reflects more the capacity of the corium to retain absorbed material than it shows true rate of absorption. This conclusion is supported by the fact that a somewhat higher amount of radioactivity could be demonstrated in the recipient portion of the chamber when it was filled with blood instead of saline.

It was concluded that parathion is very slowly absorbed, which makes this compound very unsuitable as a model substance in studies of the process of percutaneous absorption. The results have been discussed with special regard to experimental errors involved and to practical implications.

RÉSUMÉ

Il a été possible d'estimer l'absorption percutanée du parathion (E 605 ou diéthyl 4-nitrophényl thiononophosphate) *in vivo* chez le chat, en suivant la disparition d'une couche de ce produit, marqué au P^{32}, appliqué localement sur la peau (50 μl appliqué sur 4,1 cm^2). On a trouvé que cette absorption était très lente, approximativement 5 mμ M min^{-1} cm^{-2}, ce taux est près de la limite de sensibilité de la méthode et ne représente qu'environ la 25ème partie de celle du sarin. Chez deux chats, on a trouvé une nette tendance à l'inhibition de l'activité de la cholinestérase plasmatique alors qu'un troisième ne présentait pas ce phénomène. 5 heures après l'application, le taux de la radioactivité du sang s'élevait à 0,02 à 0,04 μg de parathion par ml. On a trouvé que cette technique «disparition de la substance appliquée sur la peau» n'était pas assez

sensible pour l'étude de l'absorption percutanée chez l'homme, sauf si la barrière épidermique a été lésée avec des bandes adhésives (strip-test). Le taux d'absorption a aussi été étudié *in vitro*, avec la peau excisée de l'homme, du chat et du rat, en déterminant la radioactivité dans la fraction résiduelle d'une chambre d'absorption. Chez le chat, le taux d'absorption à travers la peau excisée est beaucoup plus lent que celui de l'absorption normale *in vivo*, et on a pu en conclure que la méthode *in vitro* représente plutôt la capacité du chorion de retenir le produit absorbé que le vrai taux d'absorption. Cette conclusion est étayée par le fait que la radioactivité dans la fraction résiduelle de la chambre d'absorption est plus élevée si celle-ci est remplie avec du sang, plutôt qu'avec une solution saline. On peut conclure de cette étude que le parathion est absorbé très lentement, ce qui fait que ce produit est inutilisable en tant que substance modèle, pour l'étude des processus d'absorption percutanée. Ces résultats sont discutés, en tenant compte spécialement des erreurs expérimentales possibles et de leurs applications pratiques.

ZUSAMMENFASSUNG

Es wurde die percutane Resorptionsrate von Parathion (E 605, Diäthyl-4-nitrophenyl-thiononophosphat) *in vivo* bestimmt, indem die Abnahme der Aktivität eines lokalen Depots von P^{32}-markiertem Material (50 μl auf 4,1 cm^2) bei der Katze verfolgt wurde. Die Resorption erfolgte sehr langsam und lag mit ungefähr 5 $m\mu M\,min^{-1}\,cm^{-2}$ nahe der Empfindlichkeitsgrenze der Methode. Die Rate betrug nur etwa $^1/_{25}$ derjenigen von Sarin. Bei 2 Katzen fand sich eine deutliche Tendenz zur Hemmung der Cholinesterase-Aktivität des Plasmas; während bei einer 3. Katze dieser Befund nicht zu erheben war. Die Menge an Radioaktivität im Blut entsprach 5 Std. nach der Applikation einer Menge von 0,002—0,04 Parathion pro ml.

Diese die Abnahme der Radioaktivität verfolgende Technik erwies sich als zu wenig empfindlich, um die percutane Resorption bei Menschen zu bestimmen, es sei denn, dass die Epidermis mit Klebstreifen verletzt war.

Die Resorptionsrate wurde weiterhin *in vitro* mit excidierter Haut von Menschen, Katzen, Ratten durch Bestimmung der Radioaktivität eines Gewebsstückes in einer Resorptionskammer verfolgt. Die Aufnahme durch excidierte Haut war bei der Katze wesentlich geringer als *in vivo*, und es wurde angenommen, dass die *in vitro*-Methode mehr die Fähigkeit des Coriums, resorbiertes Material zurückzuhalten, wiedergibt als die wahre Resorption. Diese Annahme wird unterstützt durch die Tatsache, dass eine grössere Radioaktivität nachgewiesen werden konnte, wenn die Resorptionskammer anstatt mit physiologischer NaCl-Lösung mit Blut gefüllt wurde.

Es wurde gefolgert, dass Parathion sehr langsam resorbiert wird. Diese Verbindung scheint daher als Modellsubstanz zum Studium der percutanen Resorption weitgehend ungeeignet zu sein. Die Ergebnisse wurden besonders im Hinblick auf experimentelle Fehler und praktische Konsequenzen diskutiert.

RESUMEN

Ha sido determinada la velocidad de absorción percutánea del paration (E 605, o dietil 4-nitrofenil tiononofosfato) controlando la desaparición de un depósito local de material marcado con ^{32}P (50 μl aplicadas en 4,1 cm^2) en el gato. La velocidad de absorción fué muy lenta, unos 5 $m\mu M\,min^{-1}\,cm^{-2}$, lo que se aproxima al límite de sensibilidad del método y un veinticincoavo de

serina. Había una tendencia manifiesta a la inhibición de la actividad de la colinesterasa del plasma en dos gatos, que no se percibió en un tercer animal. La cantidad de radioactividad en la sangre a las cinco horas de la aplicación corresponde a 0,02 hasta 0,04 μg de paration por ml.

La técnica de desaparición se mostró carente de suficiente sensibilidad para determinar la velocidad de absorción percutánea en el hombre, salvo en los casos en que la barrera epidérmica hubiese sido lesionada con esparadrapo.

Se estudió igualmente la velocidad de absorción *in vitro* con piel escindida de hombre, gato y rata, determinando la cantidad de radioactividad en la parte receptora de una cámara de absorción. La velocidad de absorción a través de la piel escindida fué mucho menor que la de absorción general *in vivo* en el gato, concluyendo que el método *in vitro* refleja mejor la capacidad del corion para retener el material absorbido mostrando así el verdadero ritmo de absorción. Esta conclusión es reforzada por el hecho de haber podido hallar una cantidad algo mayor de radioactividad en la parte receptora de la cámara cuando se llenaba con sangre en vez de solución salina.

Se sacó la conclusión de que el paration se absorbe muy lentamente, lo que significa que este compuesto es muy inadecuado como sustancia modelo para los estudios del proceso de absorción percutánea. Los resultados han sido discutidos con miras especialmente a los errores experimentales y a las deducciones prácticas.

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PB 280 571

Reprinted from the Archives of Environmental Health
August 1961, Vol. 3, pp. 185-188
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PUBLICATION 185

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Technology Branch
Communicable Disease Center
Atlanta 22, Ga.

Percutaneous Absorption of Parathion and Paraoxon

IV. Decontamination of Human Skin from Parathion

TORSTEN FREDRIKSSON, M.D.

ATLANTA

The risk of contamination of the skin surface with radioactive materials [1] or readily absorbed chemical warfare agents [2-5] has made decontamination of human skin an important problem within the field of preventive medicine. The increasing occupational exposure to various insecticides among agricultural spray operators, crop dusters, pest control operators, and workers in formulation plants also contributes to this problem, but this is evidently less recognized. In this connection, parathion is for several reasons the most important material within this group of toxicants. It is absorbed through intact skin [6-9]; the dermal route is generally regarded as being particularly important in many cases of accidental intoxication,[10-12] and the chance for contamination of the skin among occupationally exposed persons is great.[10,13]

The aim of the present work was to investigate the efficiency of ordinary washing with soap and water to remove P^{32}-labeled parathion from the skin surface of human volunteers. The test was not mainly designed as an attempt to find effective means of decontamination or to develop a highly reproducible experimental technique, but rather to point out the existence of a serious problem in occupational medicine. In order to evaluate the importance of the interval between contamination and washing, it was decided to perform 2 series of experiments, parathion being left on the skin for 30 and 300 minutes, respectively, before decontamination. It was decided to apply the material as a 2% emulsion in xylene and water to approach actual field conditions.

Submitted for publication Feb. 16, 1961.

Dr. Fredriksson was a Visiting Scientist. Present address: Department of Dermatology, Karolinska Sjukhuset, Stockholm.

From the Toxicology Section, Technology Branch, Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta.

Methods

P^{32}-labeled parathion (E 605, diethyl 4-nitrophenyl thionophosphate) with an initial specific activity of 1.75 mc. per millimol was obtained from Volk Radiochemical Co., Chicago. It was diluted with nontagged parathion to a final activity of about 0.03 μc. per milligram. The compound was tested for contaminants by autoradiography of paper chromatograms, as described earlier.[14] Less than 0.1% of P^{32}-containing contaminants was found. An emulsion was prepared, containing 2% parathion, 2% xylene, 95.9% tap water, and 0.1% of an emulsifying agent (Triton X 100, manufactured by Rohm and Haas Co., Philadelphia). Of this emulsion, 0.1 ml., containing approximately 2.5 mg. parathion, was fairly evenly distributed with a glass rod over an area of about 12 sq. cm. on the back of the left hand of each volunteer and was left to dry with the hand in horizontal position. Two series of experiments were performed, each employing 4 men. The material was left on the skin for 30 and 300 minutes, respectively, before decontamination.

A Nuclear-Chicago D 34 thin mica (1.4 mg. per square centimeter) GM tube, operated at 950 volts, was used as a detector in conjunction with a Nuclear-Chicago binary scaler, model 162. The initial level of radioactivity was determined by inserting the contaminated hand below the detector in a lead-shielded compartment, adjusted so that movements of the hand were restricted and so

that the hand could be reinserted in approximately the same position. The activity was determined during a 1-minute period every 5 minutes in the short-term experiment. In the long-term series, the activity was determined twice at the beginning of the experiment and twice immediately before decontamination. In the long-term series, the volunteers resumed their ordinary laboratory or office work between the countings, the contaminated area of the hand being unprotected. The background activity was determined as the arithmetic average of 10-minute counts taken immediately before and after each experiment. Background counts were taken also with the right hand of each volunteer both during and after the actual experiments.

At the end of the exposure period, the volunteers were instructed to wash their hands in their usual manner, not paying any particular attention to the contaminated area. The period of washing was timed to 30 seconds. Running tap water of an approximate temperature of 37 C was used, together with an ordinary sodium soap, furnished by the Federal Supply Service and having a pH of 10 in a 5% solution. The hands then were dried thoroughly with a towel, and the residual radioactivity determined. Next, the contaminated area was washed for 15 seconds with surgical gauze sponges soaked in 95% ethanol of room temperature, and a new count taken. Finally, the 30-second hand wash with soap and water was repeated, this time with deliberate attention to the contaminated area, and any remaining activity was determined.

Results

In all cases, the area where the parathion emulsion was applied appeared to be dry within 5 to 8 minutes. There was never any visible residue, and there were no signs of irritation or any subjective sensations from the area involved. The only evidence of contamination with parathion was its characteristic odor, which was quite evident.

The results obtained from measurements of radioactivity are summarized in Tables 1 and 2. In the short-term experiments there was no measurable decrease in radioactivity during the 30 minutes preceding the decontamination. The residue after the various washings, therefore, was calculated as per cent of the arithmetic mean of the 5 countings taken during this period after subtraction of the background count. In all cases, the variation of these 5 counts was less than 4%, showing that the contaminated hands could be reinserted in the compartment in about the same position. In spite of the fairly low background (24-31 cpm) in both series of background determinations, the accuracy of the present method did not allow determination of residue activity less than 1% to 2% of the initial radioactivity, which in most cases gave 800-900 cpm. The final residue, i.e., after the second handwash, is therefore rather uncertain in 2 cases (Table 1).

In the long-term series of experiments in which the deposit was on the skin for 300 minutes, there was a decrease in radioactivity during the exposure period. The decrease was around 30% in 2 persons who continued with their laboratory duties, and

TABLE 1.—*Residual Radioactivity After Decontamination of Human Skin 30 Minutes After Application of P^{32}-Labeled Parathion, Expressed As Per Cent of Initial Activity (Initial Counting Rate 800-900 CPM)*

Volunteer No.	Residual Radioactivity, %		
	After First Handwash	After Alcohol Wash	After Second Handwash
1	8	3	<2
2	20	6	3
3	19	8	3
4	10	4	<2

TABLE 2.—*Residual Radioactivity After Decontamination of Human Skin 300 Minutes After Application of P^{32}-Labeled Parathion, Expressed as Per Cent of Remaining Activity Before Decontamination (Counting Rate Immediately Before Decontamination 600-800 CPM)*

Volunteer No.	Residual Radioactivity, %		
	After First Handwash	After Alcohol Wash	After Second Handwash
1	36	10	6
2	48	13	9
3	33	11	6
4	48	13	7

around 10% in the other 2 persons who did office work mainly. For this reason, the residue activity in this series was calculated as per cent of the arithmetic mean of the 2 counts taken immediately before the decontamination, and also, in this case, after subtraction of the background count. From Tables 1 and 2 it is evident that the decontamination procedures, particularly the first handwash, are considerably less effective when the toxic material has been left on the skin for a longer period of time. In this series, the final residue is considerable. Even when the residue in the long-term series was calculated as percentage of initial activity, the remaining percentage was considerably higher than the corresponding values for the short-term experiment.

Comment

Washing with ordinary soap and water for 30 seconds (which, in the opinion of all the volunteers, is a considerably longer period of time than they usually devote to this procedure) evidently is not particularly effective in removing parathion from the skin surface. This is especially true when the material has been left on the skin for several hours before decontamination. Furthermore, the residue might be greater than the actual figures reveal, as the self-absorption of the β-rays will increase when the compound penetrates into the skin, resulting in a lower count. There are several possible explanations for the time factor. Part of the applied material might be hydrolyzed to form diethylphosphate, which, being a moderately strong acid, might combine with proteins in the skin. However, earlier investigations [14] give no reasons to believe that this or other forms of metabolism occur to any considerable extent before the material is absorbed into the blood stream. Another explanation might be that parathion itself has been sorbed to proteins or dissolved in fats to a greater extent. The most reasonable explanation is that the compound has penetrated deeper into the skin, particularly into follicles, which makes it more difficult to elute. As has been shown in another work in this series,[15] parathion tends to accumulate in such structures after percutaneous application.

Parathion is poorly soluble in water, and this fact contributes to the low efficiency of ordinary washing. The alcohol wash, which in this case was used because parathion is readily soluble in ethanol, gave far from complete decontamination under the present experimental conditions. Even after the final handwash, there was a considerable residue, at least in the long-term experiment. Under actual field conditions, much less vigorous decontamination procedures probably are used, and the time that elapses between the initial contamination and the washing of the skin frequently is several hours, which makes the decontamination less effective. The practical implication of these factors is that occupationally exposed workers, who consistently get their body surface contaminated,[10,13] will continuously absorb parathion through the skin. Of course, not all of the deposited material will be ultimately absorbed. Part will evaporate in spite of the low vapor pressure, and part will disappear due to various external influences, as the second series of experiments indicated. There will, however, be an insidious absorption of considerable amounts, and this absorption can be assumed to continue 24 hours a day as long as the workers are exposed to the material. Even if the amount absorbed per unit time is rather small,[16] the cumulative toxicity of parathion could make this constant form of exposure an occupational health hazard. Among other things, the present findings may partly explain the fact that signs of intoxication with parathion frequently occur late in the daily exposure period or even after recognized exposure has stopped. The essentially continuous absorption may also be a factor in explaining the gradual depletion of blood cholinesterase values during a season of exposure.[17] It is my opinion that the number of intoxications due to parathion might be reduced with better and more controlled decontamination procedures.

Summary

An investigation has been made of the efficiency of a decontamination procedure in removing P^{32}-labeled parathion (E 605, diethyl 4-nitrophenyl thionophosphate) from the skin surface of human volunteers. Two series of experiments were performed, the material being left on the skin for 30 and 300 minutes, respectively. The radioactivity was determined before and after the cleansing procedure, and the residue calculated as percentage of the initial count after subtraction of the background count.

In the long-term group, ordinary washing with soap and water for 30 seconds left a residue of about 30% to 50%, while the residue amounted to 8% to 20% in the short-term experiment. An alcohol wash immediately after the initial cleansing still left a considerable residue—more than 10% in the 300-minute series and approximately 5% in the other series. A final cleansing with soap and water left a residue of more than 5% in the long-term series, while in the other series, the skin was almost completely decontaminated. The theoretical aspects of the time factor involved have been discussed, and the practical implications within the field of occupational medicine have been stressed.

Torsten Fredriksson, M.D., Department of Dermatology, Karolinska Sjukhuset, Stockholm 60, Sweden.

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 Printed and Published in the United States of America

PB 280 572

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TOXICOLOGY PROGRAM
Technology Branch
Communicable Disease Center
Atlanta 22, Ga.

Exposure to Parathion

J. D. ARTERBERRY, M.D.,
WILLIAM F. DURHAM, Ph.D.
JOSEPH W. ELLIOTT, M.S.

AND

HOMER R. WOLFE, B.S.
WENATCHEE, WASH.

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October 1961, Vol. 3, pp. 476-485
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Exposure to Parathion

Measurement by Blood Cholinesterase Level and Urinary p-Nitrophenol Excretion

J. D. ARTERBERRY, M.D.
WILLIAM F. DURHAM, Ph.D.
JOSEPH W. ELLIOTT, M.S.
AND
HOMER R. WOLFE, B.S.
WENATCHEE, WASH.

The Yakima, Okanogan, and Columbia River Valley areas of central Washington are areas of intense agricultural activity in which a wide range of pesticides finds use on a variety of crops. In these areas, parathion has been the most widely used of the more toxic insecticides. Studies of the occupational and environmental hazards associated with the use of parathion and other organic phosphorus insecticides have been reported previously.[1,2] In addition, selected occupational groups, such as insecticide spraymen[3] and crop workers,[4] have received specific study.

The decision to resurvey, during the 1958 season, several of the occupational groups studied previously in 1951 and in 1952-1953 was based on 3 factors:

1. Higher concentrations of parathion as well as more frequent applications have become necessary as the control of certain pests has become more difficult.

2. After several years of using parathion without a serious accident, it was noted that some persons had become lax in their observance of safety precautions.

3. A sensitive test for determining exposure to parathion by measuring urinary excretion of the parathion metabolite, *p*-nitrophenol, has been developed in this laboratory.[5]

In addition to the planned survey of occupational groups, a rather large number of illnesses allegedly due to parathion poisoning were encountered in central Washington during the summer of 1958. Reports of studies on these illnesses also are included.

Submitted for publication May 9, 1961.

From the Toxicology Section, Technology Branch, Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare.

Present address: Department of Pharmacology, University of Southern California, School of Medicine, Los Angeles 33 (Dr. Arterberry); Salmon Culture Laboratory, Box 410, Route 1, Entiat, Wash.

Materials and Methods

Planned Survey.—The procedure for the 1958 work was similar to that which was used in the 1951 and in the 1952-1953 surveys. The subjects were again grouped according to decreasing probable exposure. The groups studied included the following: mixing-plant personnel (MPP); commercial ground applicators (CGA); part-time ground applicators (PGA); aircraft application workers (AAW); workers in orchards, especially thinners (WO); fieldmen, warehousemen, and miscellaneous workers (FWM); and residents living near orchards (RNO). The latter group included 8 wives of orchardists who occasionally handled their husbands' work clothes and 12 wives, children, and other residents with no known direct contact with spray materials. The exposure study periods were based on the best information which could be obtained regarding exposure to insecti-

cides for each individual in order to select true preexposure and maximum exposure periods. Generally, samples taken on the last day of mixing, heavy spraying, or other intensive contact with parathion were chosen to represent maximum exposure.

The planned exposure study included 115 persons. Ninety-nine blood samples were analyzed for plasma and erythrocyte cholinesterase activity, and 571 urine samples were analyzed for *p*-nitrophenol content.

Blood cholinesterase activity determinations were carried out by the electrometric method of Michel.[6] Analyses for *p*-nitrophenol content of urine samples were done by the procedure cited above.[5]

Poisoning Cases.—Of the more than 100 cases of alleged and suspected parathion poisoning investigated during 1958, it was decided on the basis of the best and most complete information available, including both clinical and laboratory study, that 71 illnesses actually represented parathion poisoning. These illnesses were classified on clinical grounds into mild or uncertain poisoning, 19 cases; moderate, 42; severe, 8; and fatal, 2. In the poisoning cases, 37 blood, and 17 acute urine samples were analyzed. Urine samples taken within 24 hours of exposure were considered as acute. In a number of cases laboratory data were not obtained during a sufficiently acute phase of poisoning to be included here.

Only 62 persons of the 71 poisoning cases fitted into the occupational categories listed above. The nonfitting cases included 2 children (who did not live near orchards), 3 workers in hop fields, 3 orchard residents who ate parathion-contaminated food, and 1 person who was poisoned twice after different exposures. Of these 9 cases, 2 were mild, 4 were moderate, and 3 were severe.

Results

Planned Survey.—The data on blood cholinesterase activity and urinary-*p*-nitrophenol excretion for all the occupational groups included in the survey are summarized in Table 1. During the preexposure period, in instances in which more than one cholinesterase or *p*-nitrophenol value was determined for an individual during an exposure period, all the pertinent values were averaged to give a mean value for the individual. These individual mean values were used to determine the range and mean for the group. During the exposure period, the *p*-nitrophenol values were calculated in the same manner. In addition, during the exposure period, the *p*-nitrophenol and cholinesterase values were also calculated on the basis of maximum change. These maximum change figures for both cholinesterase and *p*-nitrophenol were obtained by using the cholinesterase and *p*-nitrophenol value, which was obtained from each individual during his period of maximum exposure. These figures for each individual were used to determine the group range and mean.

The over-all average in the present study for the preexposure blood cholinesterase values was 0.73 ΔpH/hour for the red blood cells and 0.95 for the plasma. These average values are very similar to the preexposure erythrocyte and plasma values of 0.77 and 0.88 ΔpH/hour, obtained in 1951, and 0.71 and 0.86, recorded in 1952.

Of the exposure groups studied, only the group with the highest potential exposure to parathion (MPP) showed a definite decrease in cholinesterase level. Diminution of enzymatic activity for this group was limited to the erythrocytes. The plasma enzyme activity was within the normal range. The results of the present study differ from those previously reported in that the other exposed groups studied (CGA, PGA, AAW, and FWM) did not show diminished cholinesterase activities, plus the fact that the extreme low values noted in some individuals in past years were not seen in any of the exposed groups. It should be noted, however, that the number of postexposure cholinesterase determinations in each of these groups was small.

The excretion of *p*-nitrophenol was insignificant for all groups during the preexposure period. During the exposure period,

TABLE 1.—*Red Blood Cell (RBC) and Plasma Cholinesterase (ChE) Values (Δ pH/hour) and Urinary p-Nitrophenol (PNP) Excretion (Parts per Million) for Various Occupational Groups and Poisoning Cases as Related to Parathion Exposure, Central Washington, 1958*

	Mixing-Plant Personnel (MPP)	Commercial Ground Applicators (CGA)	Part-Time Ground Applicators (PGA)	Aircraft Application Workers (AAW)	Workers in Orchards (WO)	Fieldmen, Warehousemen, and Office Workers (FWM)	Residents Near Orchards (RNO)
Planned Survey, Preexposure Period							
No. of persons	34	2	20	0	0	3	7
No. of blood samples	32	1	18	—	—	3	7
RBC ChE, range	0.58—0.84	0.73	0.57—0.81	—	—	0.68—0.74	0.58—0.82
RBC ChE, mean ± S.E. †	0.74±0.01	0.73	0.71±0.02	—	—	0.71±0.01	0.73±0.03
Plasma ChE, range	0.58—1.34	0.87	0.66—1.44	—	—	0.97—1.32	0.79—0.98
Plasma ChE, mean ± S.E.	0.93±0.03	0.87	0.98±0.05	—	—	1.14±0.06	0.86±0.03
No. of urine samples	34	2	18	—	—	3	7
Urinary PNP, range	el—0.07	el	el—0.22	—	—	el	el—0.09
Urinary PNP, mean ± S.E.	el	el	el	—	—	el	el
Planned Survey, Exposure Period							
No. of persons	35	2	44	4	7	3	20
No. of blood samples	8	1	13	4	0	2	5 ‡
RBC ChE, range	0.25—0.61	0.71	0.60—0.82	0.62—0.77	—	0.69—0.73	0.54—0.86
RBC ChE, mean ± S.E.	0.47±0.04	0.71	0.73±0.02	0.70±0.03	—	0.71±0.01	0.76±0.05
Plasma ChE, range	0.49—1.22	0.89	0.64—1.34	0.84—1.19	—	0.81—0.92	0.86—1.20
Plasma ChE, mean ± S.E.	0.77±0.07	0.89	1.02±0.05	1.06±0.07	—	0.87±0.03	1.04±0.06
No. of persons contributing urine	33	2	43	4	7	3	19
Maximum PNP, range	0.14—11.3	3.2—6.3	0.13—8.0	0.30—1.1	0.10—0.72	0.0 —0.35	0.05—0.31
Max. PNP, mean ± S.E.	2.0 ±0.4	4.7±1.1	2.1 ±0.3	0.65±0.16	0.28±0.08	0.15±0.09	0.11±0.02
Total no. of urine samples	154	24	290	5	9	5	20
Individual ave. PNP, range	0.11—0.5	1.1—3.8	0.15—8.0	0.30—1.1	0.10—0.70	0.06—0.26	0.06—0.31
Individual ave. PNP, mean ± S.E.	1.2 ±0.1	2.5±0.3	1.6 ±0.1	0.57±0.15	0.26±0.06	0.11±0.05	0.11±0.02
Poisoning Cases, Mild-to-Moderate							
No. of cases investigated	1	0	28	1	23	2	0
No. of cases analyzed	1	—	17	1	13	2	—
No. of blood samples	1	—	16 §	1	12 ‖	1	—
Acute RBC, ChE, range	0.59	—	0.12—0.96	0.72	0.14—0.67	0.38	—
Acute RBC, ChE, mean ± S.E.	0.59	—	0.47±0.06	0.72	0.42±0.05	0.38	—
Acute plasma ChE, range	0.70	—	0.35—0.89	1.19	0.23—0.90	0.90	—
Acute plasma ChE, mean ± S.E.	0.70	—	0.56±0.05	1.19	0.55±0.06	0.90	—
No. of urine samples	1	—	6	0	5	1	—
Acute PNP, range	3.2	—	0.57—32.2	—	0.57—12.7	1.0	—
Acute PNP, mean ± S.E.	3.2	—	8.4 ±2.2	—	6.0 ± 0.7	1.0	—

	Poisoning Cases, Severe-to-Fatal ¶							
No. of cases investigated	0	0	3	2	2	0	?	
No. of cases anal., red	—	—	3	2	2	—	—	
No. of blood samples	—	—	2	2	2	—	—	
Acute RBC ChE, range	—	—	0.09—0.14	0.09—0.11	0.20—0.22	—	—	
Acute RBC ChE, mean ± S.E.	—	—	0.10±0.03	0.10±0.01	0.21±0.01	—	—	
Acute plasma ChE, range	—	—	0.17—0.19	0.20—0.22	0.20—0.39	—	—	
Acute plasma ChE, mean ± S.E.	—	—	0.18±0.01	0.21±0.01	0.33±0.05	—	—	
No. of urine samples	—	—	2	—	2	—	—	
Acute PNP, range	—	—	8.4 —9.5	—	1.6 —11.6	—	—	
Acute PNP, mean ± S.E	—	—	9.0 ±0.4	—	6.6 ± 3.6	—	—	

* Only 5 plasma values available
† Standard error of the mean
‡ Only 4 plasma values available.
§ al Value less than experimental limit of the chemical method
§ Only 12 plasma values available.
‖ Only 10 plasma values available.
¶ Of the 71 cases studied, 9 did not fit into the occupational categories listed.

p-nitrophenol excretion was well related to occupational parathion exposure. All 5 of the highest exposure groups (MPP, CGA, PGA, AAW, and WO) excreted significant quantities of *p*-nitrophenol. The excretion by the 3 highest exposure groups (MPP, CGA, and PGA) was of a similar order of magnitude. The excretion of measurable amounts of *p*-nitrophenol in the presence of normal plasma and red blood cell cholinesterase levels is important as an indication that *p*-nitrophenol excretion is a more sensitive measure of exposure to parathion than is blood cholinesterase activity. The excretion of the fieldmen, warehousemen, and miscellaneous workers group (FWM) was quite low and of questionable significance. The average excretion of the resident near-orchard group was not significant. Only one sample from one individual, in this group contained a significant quantity of *p*-nitrophenol. Thus, this more sensitive measure of exposure essentially confirms results with blood cholinesterase tests and symptomatology, since neither of the latter 2 criteria have indicated significant exposure of the environmentally exposed group either in the present study or in the 2 previous surveys.

The plasma cholinesterase value for one individual in the RNO group was eliminated from consideration in Table 1. This value was found to be unusually high from blood samples taken on the 2 different occasions when she was tested. The subject, the 70-year-old wife of a local orchardist, had values of 2.30 and 2.80 ΔpH/hour. These values are significantly higher than any maximum range for plasma cholinesterase previously reported in the literature. Her red cells gave values of 0.80 and 0.88 ΔpH/hour which are, of course, well within the range of average normal activity. This woman is in excellent health and, in fact, claimed not to have consulted a physician for 20 or 30 years. She gave no history of intake of thyroid, estrogen, or other hormones. She ate no special foods and had no signs or symptoms of disease. Laboratory studies carried out through the

cooperation of the U.S. Public Health Service Hospital, Seattle, were normal, showing blood urea nitrogen, 13.6 mg. %; albumin, 4.17 gm. %, and globulin, 2.56 gm. %. By paper electrophoresis the following values for blood protein were obtained: total protein, 7 gm. %; albumin/globulin ratio, 1.74/1.00; albumin, 64%; αI-globulin, 4.1% (2.4-5.3); αII-globulin, 7.6% (6.6-13.5); β-globulin, 8.6% (8.5-14.5); and γ-globulin, 16.2% (10.7-21.0). All of these values are within the normal range for the general population.

Poisoning Cases.—Two fatalities attributed to parathion poisoning occurred in central Washington during 1958. Both men were employed as part-time ground applicators. In one of these cases, occupational exposure of an untrained sprayman was apparently responsible for the fatality. In the other case, although the man was occupationally exposed, ingestion of parathion (accidental or intentional) was suspected to have occurred.

The data on erythrocyte and plasma cholinesterase levels and urinary *p*-nitrophenol excretion for the poisoning cases studied are given in Table 1. The cases were classiged as mild-to-moderate or severe-to-fatal solely on clinical grounds.

It will be noted that at least one poisoning case was encountered in men representing each of the occupational groups having significant occupational exposure to parathion, with the exception of the commercial ground applicators, who comprise a much smaller group, numerically, than any of the others. No case of poisoning was seen in persons with environmental exposure only. Although cholinesterase depression and *p*-nitrophenol excretion corresponded to the degree of occupational exposure among the several groups, the number and severity of poisoning cases showed no such correlation. One is tempted to read into this finding support for the hypothesis that, regardless of the degree of his expected occupational exposure, almost any person who works with parathion is in danger of being poisoned if he becomes careless.

The erythrocyte and plasma cholinesterase levels for both the mild-to-moderate and the severe-to-fatal cases considered as a whole were depressed from the normal level. The severe-to-fatal group was depressed significantly more than the mild-to-moderate group. The average erythrocyte enzyme level was 0.46 ΔpH/hour for the mild-to-moderate group and 0.14 for the more severely poisoned cases. The plasma activity levels were 0.60 and 0.24 ΔpH/hour, respectively, for the 2 groups. As in the past, however, there were some cases diagnosed as mild or moderate poisoning which did not show a cholinesterase activity lower than the average range of normal. (The individual normals were not known in the present cases.) It should be noted that even these latter cases showed significant excretion of *p*-nitrophenol. All the severe-to-fatal cases had significant depletion of enzyme function as well as positive values for *p*-nitrophenol excretion.

All the poisoning cases studied in this regard excreted easily detectable quantities of *p*-nitrophenol, ranging from 0.57 to 32.2 ppm for the mild-to-moderate cases and from 1.6 to 11.6 for the severe-to-fatal cases. It is interesting to note that the highest value seen (32.2 ppm) was in a case of moderate severity. Although the average *p*-nitrophenol excretion was somewhat higher for the severe-to-fatal cases (7.8 ppm) than for the mild-to-moderate ones (6.5 ppm), it is apparent from the above discussion that *p*-nitrophenol excretion was not a reliable measure of the severity of poisoning.

Pharmacodynamics of p-*Nitrophenol Excretion.*—Following exposure to parathion, the excretion of *p*-nitrophenol in the urine is quite rapid, as might be expected in the case of a water-soluble metabolite of a substance which is quickly broken down by the animal organism. *p*-Nitrophenol usually has disappeared from the urine within about 48 hours after cessation of exposure. This finding is illustrated by Curves 1 and 2 in Figure 1. In view of this rapid excretion of *p*-nitrophenol, the importance of

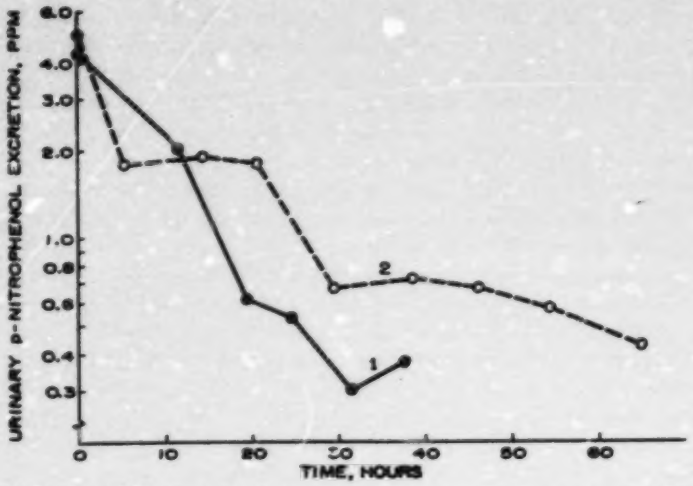

Fig. 1.—Urinary excretion of *p*-nitrophenol in 2 individuals following heavy exposure to parathion.

obtaining samples soon after exposure is evident. Samples taken 48 hours or more following exposure are essentially useless for this purpose.

Figure 2 shows the urinary *p*-nitrophenol excretion levels of an orchard worker who was exposed to rather large amounts of parathion for the indicated number of hours each day over a period of several days while mixing the spray formulation used in a large orchard. It can be seen that the *p*-nitrophenol excretion increased immedi-

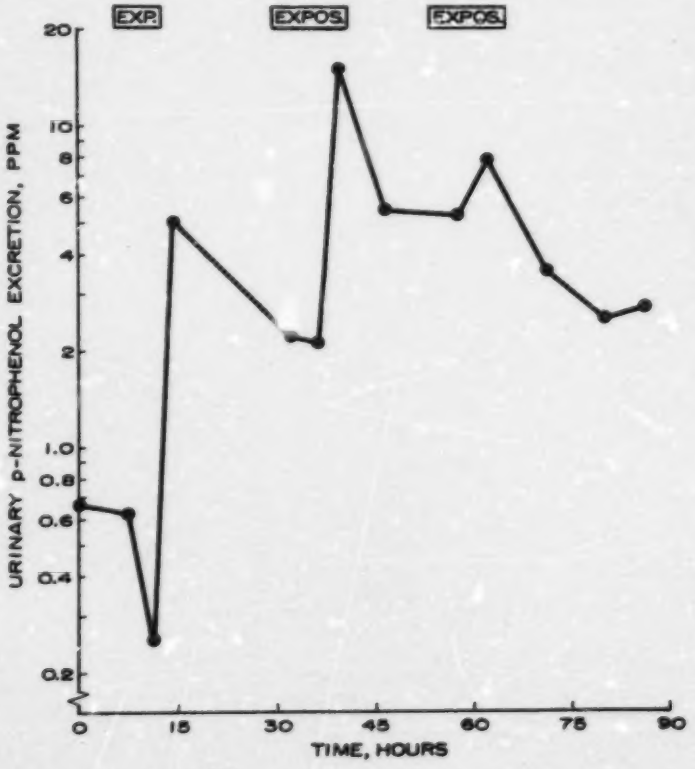

Fig. 2.—Urinary *p*-nitrophenol excretion of a worker who mixed parathion formulation for use in a large orchard. Exposure periods are indicated.

ately with each exposure period, but diminished appreciably during the period between exposures. The rate of excretion after exposure was approximately the same as that in the poisoning cases.

Relationship Between Blood Cholinesterase Level and Urinary p-Nitrophenol Excretion.—The relationship between blood cholinesterase level and urinary *p*-nitrophenol excretion for survey groups and for poisoning cases is shown in Figure 3. There is only a very gross correlation between plasma and erythrocyte cholinesterase levels and *p*-nitrophenol excretion. However, it would appear that people develop a relatively small depression of cholinesterase proportional to the concentration of *p*-nitrophenol excretion up to a certain level (about 2 ppm of *p*-nitrophenol) and a more pronounced depression proportional to higher concentration of *p*-nitrophenol excretion.

Unfortunately, no poisoning cases were studied in which both blood and urine samples were available in the acute phase of illness and during convalescence. Therefore, no data are available to show the simultaneous variations in *p*-nitrophenol excretion and blood cholinesterase level in the same individual during acute poisoning and recovery. However, the changes in blood cholinesterase level associated with poisoning are well established. After severe poisoning, the blood cholinesterase values remain depressed for about 30 days (plasma) or 80-100 days (erythrocytes). By contrast, *p*-nitrophenol excretion falls to near zero in only a day or two after a single heavy exposure (Fig. 1) and even falls

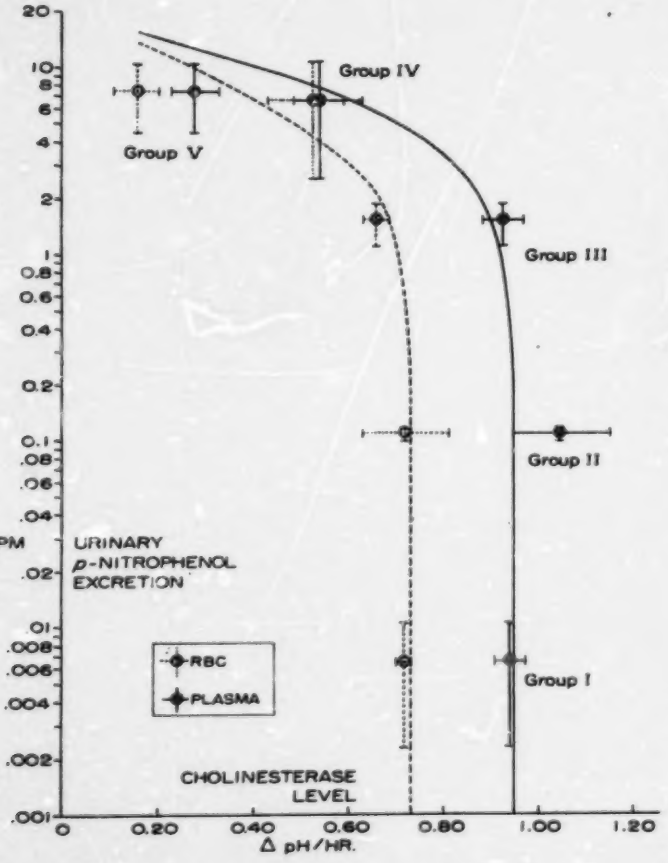

Fig. 3.—Relationship between blood cholinesterase levels and urinary *p*-nitrophenol excretion for occupational groups and poisoning cases, central Washington, 1958. Group I, planned survey, pre-exposure period (56 cases); Group II, residents near orchard during exposure period (3 cases); Group III, planned survey, exposure period (25 cases); Group IV, poisoning cases, mild to moderate (7 cases); Group V, poisoning cases, severe to fatal (3 cases). Each point represents the mean and associated standard error for a group of cases. Only cases for which both cholinesterase and *p*-nitrophenol values were known are included.

TABLE 2.—*Occurrence of Various Selected Signs and Symptoms in 71 Cases of Illness Diagnosed as Parathion Poisoning in Central Washington in 1958*

Sign or Symptom	Severity of Illness				
	Fatal	Severe	Moderate	Mild or Uncertain	Total
Cardiovascular					
Hypothermia	--*	1	1	--	2
Irregular cardiac rate	2	2	--	--	4
Shock or impending shock	2	2	--	--	4
Tachycardia	1	2	1	1	5
Gastrointestinal					
Abdominal pain, distention, tenderness	--	3	16	4	23
Anorexia	--	1	11	--	12
Diarrhea	--	4	17	2	23
Nausea	1	7	36	12	56
Sialorrhea	2	1	--	2	5
Vomiting	1	7	23	5	36
Weight loss	--	--	4	--	4
Neurologic					
Apprehension	1	2	1	--	4
Ataxia	--	3	5	--	8
Convulsions	1	--	--	1	2
Drowsiness	--	--	1	4	5
Fasciculations	--	2	2	--	4
Giddiness	--	--	2	--	2
Headache	--	2	20	4	26
Loss consciousness	2	2	3	--	7
Mental confusion	--	4	5	1	10
Paresthesia	--	--	1	1	2
Vertigo	--	4	10	2	16
Ophthalmologic					
Eye pain	--	--	--	5	5
Fluttering eyelids	--	--	4	2	6
"Glassy" eyed stare	1	1	--	--	2
Miosis, visual disturbance	--	5	15	6	26
Respiratory					
Chest pain, severe	--	1	7	3	11
Cough	--	--	4	--	4
Cyanosis	2	3	1	--	6
Hoarseness, aphonia	--	--	3	1	4
Pulmonary edema	2	3	--	2	7
Respiratory difficulty	2	3	11	2	18
Miscellaneous					
Excessive perspiration	2	1	10	1	14
Fatigue	--	4	25	7	36
Frequency, urgency	--	1	2	--	3
Good atropine response	--	7	11	3	21
Leukocytosis	1	2	2	--	5
Malaise	2	4	20	8	34
Nasal discharge, stuffiness	--	--	2	3	5
Rash	1	--	2	--	3
Spasm or joint pain	1	1	5	2	9
Total cases	2	8	42	19	71

* One case had ventricular fibrillation.

sharply during the intervals between daily exposures (Fig. 2). It should be noted that the rapid disappearance of *p*-nitrophenol following cessation of exposure makes this test of considerable value in evaluating an acute situation in a chronically exposed individual.

Symptomatology.—The symptomatology of the various exposure groups has been studied in the 2 previous surveys[1,2] carried

out in north central Washington by this laboratory. This facet of the older work was not repeated. However, the occurrence of various signs and symptoms among the group of poisoned individuals has been carefully analyzed and tabulated with regard to clinical degree of illness. In Table 2 are shown the most prominent signs and symptoms displayed by the 71 poisoning cases studied in 1958.

The predominance of gastrointestinal complaints was noteworthy. The most commonly reported symptom was nausea, which occurred in 56 individuals. Among the other more common complaints were vomiting (36 cases); fatigue (36 cases); malaise (34 cases); miosis or visual disturbance (26 cases); headache (26 cases); diarrhea (23 cases); abdominal pain, distention, or tenderness (23 cases); and respiratory difficulty (18 cases). A number of closely related neurological conditions were seen, including giddiness (2 cases), ataxia (8 cases), drowsiness (4 cases), paresthesia (2 cases), and loss of consciousness (6 cases).

A sense of fulness or tightness in the chest has been recognized as one of the expected symptoms of parathion intoxication. However, the rather severe anginal-type pain reported by 11 cases in this survey was considered by some physicians to be different from this earlier-reported syndrome. The chest pain was similar in some respects to that of myocardial infarction. In 3 cases the latter diagnosis was not substantiated by electrocardiogram and, in the remaining cases, was ruled out on a clinical basis by the disappearance of pain shortly after injection of atropine.

Leukocytosis occurred in all 5 cases in which a white cell count was reported.

A good response to atropine was the most reliable clinical criteria of parathion poisoning. A good response was noted in 21 out of the 23 persons to whom this medication was given. Only 1 of these 23 persons had absolutely no response.

Comment

The observed rapid rate of *p*-nitrophenol excretion was in accord with the results of Gardocki and Hazelton,[7] who recovered up to 77.7% of intravenously injected parathion as urinary *p*-nitrophenol from dogs during the 8 hours after dosing. However, Lieben et al.[8] reported a single dose of parathion given to monkeys, either subcutaneously or by skin application, resulted in a prolonged excretion of *p*-nitrophenol in the urine, lasting up to 32 days. The explanation for the very long excretory period observed by Dr. Lieben and his co-workers is not immediately apparent.

Urinary *p*-nitrophenol excretion has both advantages and disadvantages over blood cholinesterase level as an indicator of poisoning by parathion. Among the advantages of the *p*-nitrophenol test are its greater sensitivity and the increased patient cooperation in providing urine samples as compared to blood samples. Its disadvantages include the fact that the test is informative for a rather short period of time (about 48 hours) following exposure. The specificity of the test, since it is limited to parathion and EPN among the more commonly used insecticides, could be either an advantage or a disadvantage, depending, for example, on whether one wished to pinpoint the parathion exposure of a worker also exposed to other phosphorus compounds, or to determine a sprayman's total exposure to a number of these materials.

It appears, on the basis of the results presented above, that urinary *p*-nitrophenol excretion is an excellent indicator of exposure to parathion and is significantly more sensitive in this regard than is blood cholinesterase level. However, blood cholinesterase level shows a better correlation with the occurrence of selected symptoms in exposed individuals and with severity of poisoning in parathion-induced illness than does *p*-nitrophenol excretion. These findings are consistent with the idea that changes in blood cholinesterase level are a function of the pharmacologic effect of the

poison on the organism, while *p*-nitrophenol is not a measure of effect but represents the amount of toxicant which the organism has absorbed and metabolized. The difference in reliability between these 2 methods as criteria of poisoning may be a reflection of the variability between individuals in susceptibility to the toxicant.

The occurrence of rather low urinary *p*-nitrophenol values in poisoned individuals and, more particularly, of high values in apparently well people emphasizes the critical time factor in collecting urine samples plus the unreliability of *p*-nitrophenol excretion as the sole criterion for a diagnosis of parathion poisoning. Rather, *p*-nitrophenol excretion should be used as a substantiation of exposure and the presence or absence of poisoning should be judged on a clinical basis with the guidance of blood cholinesterase activity levels.

Summary

A survey of blood cholinesterase activity and urinary *p*-nitrophenol excretion of occupational groups exposed to parathion was made in central Washington in 1958. Only the group with the highest potential exposure to parathion (mixing-plant personnel) showed a definite decrease in cholinesterase level during exposure. The enzyme diminution for this group occurred only with the erythrocytes. All 5 of the highest exposure groups (mixing-plant personnel, commercial ground applicators, part-time ground applicators, aircraft application workers, and workers in orchards) excreted significant quantities of *p*-nitrophenol during the period of their exposure. The excretion of measurable amounts of *p*-nitrophenol in the presence of normal plasma and red blood cell cholinesterase levels is important as an indication that *p*-nitrophenol excretion is a more sensitive measure of exposure to parathion than is blood cholinesterase activity. The average *p*-nitrophenol excretion of the residents-near-orchard group was not significant.

Blood cholinesterase levels, *p*-nitrophenol excretion, and symptomatology also were determined for a number of parathion poisoning cases. *p*-Nitrophenol excretion was elevated in all poisoning cases studied. Some mild-to-moderate cases showed no significant cholinesterase depletion, but all severe-to-fatal cases had depressed enzyme function.

The excretion of *p*-nitrophenol was quite rapid, and this metabolite had usually disappeared from the urine within about 48 hours after exposure.

A good response to atropine appeared to be the most reliable clinical criterion of parathion poisoning.

William F. Durham, Ph.D., U.S. Public Health Service, P.O. Box 73, Wenatchee, Wash.

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PB 280 573

PUBLICATION

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Acta Dermato-Venereologica, Vol. 41, p. 344–352 (1961)

TOXICOLOGY PROGRAM
Technology Branch
Communicable Disease Center
Atlanta 22, Ga.

STUDIES ON THE PERCUTANEOUS ABSORPTION OF PARATHION AND PARAOXON

II. Distribution of ^{32}P-Labelled Parathion within the Skin

TORSTEN FREDRIKSSON*
ATLANTA, GEORGIA, U. S. A.

In recent years, autoradiographic techniques frequently have been used to study the cutaneous distribution of various compounds applied topically, occasionally in order to determine routes of absorption (Witten, 1951; Scott and Kalz, 1956; Blank *et al.*, 1958; Fredriksson, 1958; Blank and Gould, 1959). However, there have been no previous investigations of this kind concerned with parathion, an organophosphorus insecticide which is known to produce occupational intoxications by percutaneous absorption, as reviewed earlier, Fredriksson *et al.*, 1961. Since this compound is of great practical importance, it was decided to study the distribution of ^{32}P-labelled parathion within the skin of various species following topical application, with special attention paid to artefacts produced by diffusion, time of exposure, and type of radiation.

Methods

^{32}P-labelled parathion (E 605, or diethyl 4-nitrophenyl thiononophosphate) with an initial specific activity of 1.75 mC per mM, furnished by Volk Radiochemical Company, Chicago, Ill., was used in all the experiments. When tested for purity (Fredriksson *et al.*, 1961) it was found to contain less than 0.1 percent of an unidentified contaminant.

Skin from man, cat, rabbit, and rat was used. Human sternal skin was obtained at autopsy, performed within 10 hours of death. These human skin samples, which were from adult individuals of both sexes, had evidently not been washed or otherwise treated and showed no evidence of disease. Skin samples were taken from a clipped area on the back of cats and rabbits under sodium pentobarbital anaesthesia. Rats (Sherman strain) were killed by a blow on the head, and immediately after death clipped skin was removed from the back.

Stripping experiments

Pieces of human skin were nailed to a board, and with the aid of a glass rod, 6 μl of labelled parathion were evenly distributed on a round area of

Received for publication May 31, 1961.

* Dr. Fredriksson was a Visiting Scientist. *Present address:* Department of Dermatology, Karolinska sjukhuset, Stockholm 60, Sweden.

Toxicology Section, Technology Branch, Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia, U.S.A., and the Department of Dermatology, Karolinska sjukhuset, Stockholm 60, Sweden.

approximately 1.5 cm^2. The treated skin was then placed in a humid chamber where the temperature was maintained at 23 to 24° C. The parathion was allowed to remain on the skin for 30 minutes and for 1, 2, 4 and 24 hours. At the end of the exposure period, the excess parathion was removed by one gentle blotting with a high grade, smooth surface, medium speed filter paper (Scientific Products, No. 63200). Twenty-five consecutive strippings then were made of the treated area with the aid of 1″ transparent cellulose tape. Each set of strips consisting of cellulose tape and attached skin, was immediately attached to a 1—1/4″ wide band of cellophane, which had a thickness of approximately 40 μ. This band was then run in a Nuclear-Chicago model C 100 A Actigraph II chromatogram scanner with the cellophane, which had a more uniform thickness than the tape, facing the detector. The scanning speed was 3″ per minute, and the slit opening was set at 1/16″. A Nuclear-Chicago model D 34 thin mica window (1.4 mg/cm^2) GM-tube, operated at 950 volts, was used as detector together with a Nuclear-Chicago recording rate meter model 1620 B, with the time constant set at 2 seconds.

With the cellophane facing the film, autoradiograms were then obtained by apposition to Eastman-Kodak No Screen Medical X-ray film. The autoradiograms were subsequently developed and fixed as described below. All experiments were run in duplicate.

Autoradiograms from sections

Skin samples from all the species were included in these experiments, and the labelled parathion was applied in the same manner and for the same exposure period as above. At the end of the exposure period, the skin samples were placed immediately on dry ice and transferred to a freeze room (— 10° C). In one series of experiments the excess parathion was left on the skin surface, and in another series it was removed by blotting with filter paper as above. The frozen pieces of skin were sectioned transversely in the freeze room at 10, 25 and 100 μ, particular care being taken to avoid radioactive contamination of the knife or the sections. The following four autoradiographic techniques were used: 1) sections were mounted directly on photosensitive slides (Eastman-Kodak Autoradiographic Plates Type A and Type No Screen); 2) sections were mounted on glass slides and autoradiograms were obtained by apposition to the same photosensitive slides as above and to Eastman-Kodak No Screen Medical X-ray film; 3) sections were attached to transparent cellulose tape, according to Ullberg, 1954, and autoradiograms were obtained by apposition as above; and 4) sections were mounted on glass slides and covered with small pieces of Eastman-Kodak Autoradiographic Safety Stripping Film Type NTB, essentially according to Boyd, 1955. Control studies were performed *in vivo* with cats and rabbits under sodium pentobarbital anaesthesia to learn whether there was any difference in the distribution of parathion in excised and intact skin; the parathion in these cases was left on the skin for 1 hour. In all cases, controls were run also with sections from skin that had not been exposed at all to labelled material. Exposure of photosensitive material took place in the freeze room or at room temperature. In the freeze room, the sections were allowed to reach temperatures above the freezing point only when they were momen-

tarily warmed against the back of the bare hand in order to make them adhere to the slides. This was not necessary when the sections were attached to the cellulose tape and brought in contact with the film under pressure. All the autoradiograms were developed with Eastman-Kodak D 19 developer for 5 minutes at 21° C, transferred to an acid stopping bath, and fixed for 30 minutes with Kodak fixing bath F 5. The final rinsing water contained 0.5 percent of Eastman-Kodak Hypo-flo solution. The sections were mounted and examined in phase contrast.

Results

Stripping experiments

The results are illustrated by Figs. 1 and 2. As can be seen from Fig. 1, there was a rapid drop in activity in the first four strips followed by a much slower but steady decrease in activity. However, even in strip number 25, the activity peak was significantly higher than the background. This general pattern was the same regardless of the exposure time.

Following relatively short exposure (2 hours or less) to the X-ray film, the autoradiograms, with the partial exception of the first three or four strips, showed an uneven distribution of the labelled material, which corresponded to the follicular pattern of the piece of skin in question. This follicular arrangement of the activity was obscured by prolonged exposure to the photosensitive film. There was a tendency towards more uniform distribution of the material when it had been in contact with the skin for 24 hours, but even in this case there was a distinct follicular arrangement.

Autoradiograms from sections

The results are illustrated by Figs. 3 and 4. The various factors studied and the procedures that influenced the autoradiograms and their interpretation are listed below.

Controls without labelled material. Occasionally, artefacts appeared that were produced by mere contact of the unexposed sections with the photosensitive material. Such darkening usually followed the border of the sections, and also showed some predilection for such structures as hair follicles and sweat glands.

Control experiments in vivo. The control studies, performed on cats and rabbits *in vivo*, did not produce autoradiograms that differed in any respect from those obtained *in vitro* from the same species and with the corresponding exposure period.

Removal of the excess parathion. When the excess parathion was not removed, the autoradiograms were obscured by an intense darkening corresponding to the skin surface, and the photosensitive material could not be exposed sufficiently long to detect the comparatively low activity at more deeply located sites. Furthermore, under this condition, radioactive contamination of the microtome knife or the sections frequently occurred. However, when the excess was removed by blotting with filter paper longer exposure of the photosensitive material was possible, and thus, a more detailed picture of the actual distribution of the labelled material could be obtained.

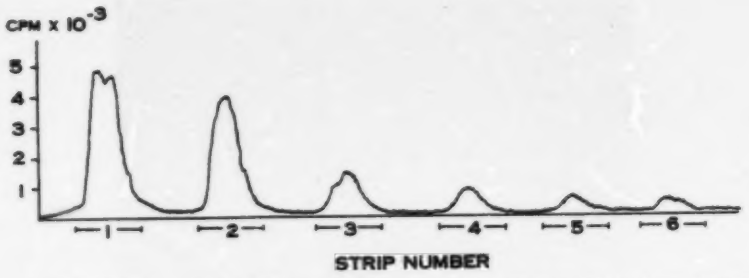

Fig. 1. Radioactivity as obtained from recorder in the first 6 tape strips from human skin, taken 1 hour following application of 6 μl of ^{32}P-labelled parathion. Scanning speed 3" per min.; slit opening 1/16"; time constant 2 seconds.

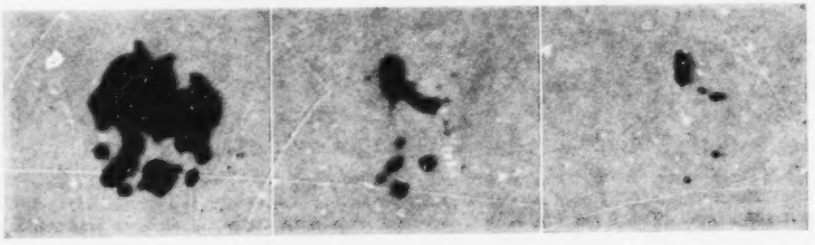

Fig. 2. Autoradiograms of strip no. 3, 10, and 25 from the same experiment as in Fig. 1. Magnification: 2 ×. Exposure of photosensitive material 2 hours.

Thickness of sections. As a rule, the most detailed pictures were obtained with the thinnest sections, which had relatively less activity on the surface, and, therefore, could be allowed longer exposure to the photosensitive material.

Temperature during exposure of photosensitive material. There were seldom any evident diffusion artefacts in the autoradiograms obtained by exposure at room temperature, probably due to the rather short time of exposure — generally less than 4 hours. However, as a precaution, only freeze-room exposed autoradiograms were used for the final evaluation. The momentary elevation of the temperature when the sections were mounted did not seem to produce any artefacts, since the sections, which were attached to the cellulose tape and which were kept at — 10° C during the whole exposure period, gave identical pictures.

Autoradiographic techniques. The four autoradiographic techniques used gave essentially the same results. Direct mounting on photosensitive slides or the use of stripping film allowed, however, much higher resolution than the apposition techniques, and autoradiograms so obtained were therefore used for the final interpretation.

Exposure time of photosensitive material. It already has been indicated that the time of exposure to the photosensitive material must be carefully selected in order to obtain accurate pictures of the distribution, particularly when there

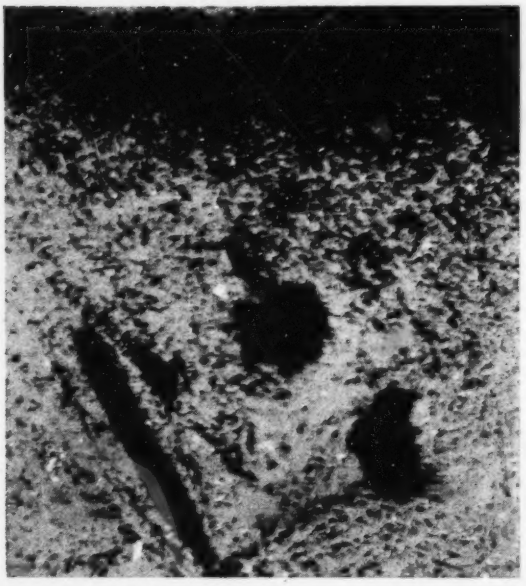

Fig. 3. Distribution of ^{32}P-labelled parathion in human skin 24 hours after application. The central structure, suggestive of a sweat gland, is in reality a hair follicle. Eastman-Kodak Autoradiographic Plate Type No Screen. Exposure of photosensitive material 2 hours. Magnification: 100 ×. Phase contrast with focusing between emulsion and section.

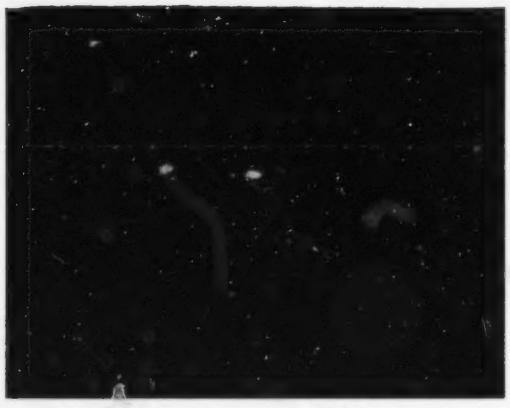

Fig. 4. Distribution of ^{32}P-labelled parathion in cat skin 2 hours from application. Two barely noticeable pressure artefacts from hairs in left part of the autoradiogram. Eastman-Kodak No Screen Medical X-ray Film. Exposure of photosensitive material 2 hours. Magnification: 5 ×.

are marked relative variations in activity in the sections. In the present case an exposure of 2 to 4 hours gave the most detailed pictures, and longer exposure than this gave no further information but rather tended to obscure the pictures due to the high activity on the skin surface.

Processing of sections. Occasionally, the development of the underlying emulsion or the processing of the tissue sections produced a noticeable shrinkage of the tissues, resulting in a relative displacement of the sites of activity. However, it was usually possible to deduce the original site of activity, even in slides with such displacement.

Final interpretation of the autoradiograms. The follicular arrangement of the labelled material, demonstrated in the stripping experiments, was not always evident in the autoradiograms from the sections, particularly when the photosensitive material had only been exposed for a short period of time. As could be expected from the stripping experiments, there was much higher activity corresponding to the skin surface than anywhere else in the sections, even when a major part of the labelled material had been removed by the blotting. However, activity could always be demonstrated in hair follicles and sebaceous glands. The amount in the follicles seemed to increase progressively with time; when the skin had been exposed to parathion for 24 hours, there also appeared to be an increased spreading around the follicles and directly below the epidermis. There seemed to be no increase in activity around sweat glands or ducts in human skin. There were no apparent species differences, even though it was easier to demonstrate the penetration into follicles in animal skin than in human skin.

Comments

It has been pointed out repeatedly that the interpretation of autoradiograms from sections of the present kind must be done very carefully, particularly when they have been obtained with a high energy β-emitter such as ^{32}P, since the particles may travel some distance before darkening the photosensitive material. (Blank *et al.*, 1958; Blank and Gould, 1959, Griesemer, 1959). Also, the present study indicates that various forms of artefacts constitute a most important limiting factor for definite conclusions regarding routes of percutaneuos absorption. It is the opinion of the present author that the pictures obtained reveal affinity of parathion for certain structures rather than actual pathways of absorption. After all, it is an obvious fact that autoradiograms of this kind show only what has *not* been absorbed.

However, the autoradiograms from the sections combined with the results obtained with the rather crude stripping method indicate that parathion penetrates into hair follicles to some extent. This can easily be overlooked in case the amount of labelled material on the surface is large and the exposure of the sections to the photosensitive material is short. The progressively increasing amounts in the follicles and the spreading around them does not necessarily mean that this is the main route of absorption, as Blank has pointed out in the discussion of a paper presented by Griesemer, 1959. The increasing radioactivity below the epidermis makes transepidermal absorption just as likely.

It does not seem probable that the blotting used to remove part of the excess

on the skin surface will produce any rearrangement of the labelled material. At least, the risks of such artefacts appear to be much less than it is when the excess is removed by washing, which sometimes is necessary with more toxic compounds like sarin (Fredriksson, 1958).

Even if no certain conclusions can be drawn from the present study regarding routes of absorption or, for that matter, the location of the epidermal barrier, the results have other immediate implications. Thus, the penetration into follicles helps to explain the shape of disappearance curves obtained by measurements of rates of percutaneous absorption (Fredriksson, 1958; 1961), and may also explain the difficulties in decontaminating skin from parathion (Fredriksson, 1961).

SUMMARY

The distribution of ^{32}P-labelled parathion (E 605, or diethyl 4-nitrophenyl thiononophosphate) within the skin following topical application for various periods of time was studied in excised skin from man, rat, rabbit, and cat. Two different approaches were chosen: 1 a) determination of radioactivity in 25 consecutive cellulose tape strips from the surface of human skin, 1 b) autoradiography of the same strips; and 2) autoradiography of skin sections with the use of four different techniques. Various factors influencing the autoradiograms were studied, special attention being paid to artefacts due to diffusion, type of radiation, and exposure time of the photosensitive material. It was found that parathion penetrates into hair follicles and sebaceous glands to some extent, but it was concluded that this is not necessarily the main route of absorption. There also was increasing activity below the epidermal layers, and transepidermal absorption is as likely. The results were discussed with special attention to the interpretation of the autoradiograms and to the implications for other studies of the percutaneous absorption of parathion.

RÉSUMÉ

La répartition du parathion (E 605 ou diéthyl 4-nitrophényl thiononophosphate) marqué au P^{32} se trouvant à l'intérieur de la peau, à la suite d'une application locale et après des temps variés, a été étudiée dans la peau excisée de l'homme, du rat, du lapin et du chat. Deux méthodes différentes ont été adoptées: 1 a) la détermination de la radioactivité de 25 strip-tests pris successivement à la surface de la peau humaine, 1 b) les autoradiographies de ces mêmes strip-tests et 2) les autoradiographies de coupes de peau, au moyen de 4 techniques différentes. Les divers facteurs susceptibles d'influencer les autoradiographies ont été étudiés, en prêtant spécialement attention aux artefacts provenant de la diffusion, au type de radiations, et au temps d'exposition du matériel photosensible. On a trouvé que le parathion pénètre dans une certaine mesure dans les follicules pileux et les glandes sébacées, mains on a pu conclure qu'il ne s'agit pas nécessairement de la voie principale d'absorption. Il existe aussi une augmentation de l'activité dans les couches sous-épidermiques qui rend l'absorption transépidermique plus facile. Ces résultats ont été discutés en portant spécialement attention à l'interprétation des autoradiogrammes et à l'utilisation de l'absorption percutanée du parathion en vue d'études ultérieures.

ZUSAMMENFASSUNG

Es wurde die Verteilung von P^{32}-markiertem Parathion (E 605, Diäthyl-4-nitrophenyl-thiononophosphat) in excidierter Haut von Menschen, Ratten, Kaninchen und Katzen in verschiedenen Zeitabständen nach der Excision untersucht. Zwei verschiedene Versuchsanordnungen wurden gewählt: 1 a) Bestimmung der Radioaktivität von 25 aufeinander folgenden, mit Hilfe eines Cellulose-Streifens vorgenommenen Abzügen der Hautoberfläche; 1 b) Autoradiographie der gleichen Abrisse; 2) Autoradiographie von Hautschnitten mit 4 verschiedenen Techniken. Verschiedene Faktoren, die die Autoradiogramme beeinflussen, wurden untersucht, wobei besonderer Wert auf Artefakte infolge Diffusion, Typ der Strahlung und Expositionszeit des photoempfindlichen Materials gelegt wurde. Es zeigte sich, dass Parathion zu einem gewissen Ausmass in die Haarfollikel und Talgdrüsen eindringt; aber es wurde geschlossen, dass dieses nicht notwendigerweise der Hauptweg der Resorption ist. Es fand sich eine gesteigerte Aktivität unterhalb der epidermalen Schichten; transepidermale Resorption ist ebenso wahrscheinlich. Die Ergebnisse werden diskutiert mit besonderer Berücksichtigung der Interpretation der Autoradiogramme und der Folgerungen für andere Untersuchungen der percutanen Resorption von Parathion.

RESUMEN

Se estudió la distribución en la piel del paration (E 605, o dietil 4-nitrofenil tiononofosfato) marcado con ^{32}P, en aplicaciones tópicas en períodos de tiempo variables, a piel escindida de hombre, rata, conejo y gato. Se han seguido dos caminos: 1 a) determinación de radioactividad en 25 tiras consecutivas de celulosa aplicadas a la superficie de la piel humana, 1 b) autorradiografía de dichas tiras; y 2) autorradiografía de secciones de piel utilizando cuatro diferentes técnicas. Se estudiaron varios factores que influyen los autorradiogramas, atendiendo especialmente a los artefactos debidos a la difusión, tipo de radiación y tiempo de exposición del material fotosensitivo. Se comprobó que el paratión penetra en cierto grado folículos pilosos y glándulas sebáceas, pero se sacó la conclusión de que éstas no constituyen forzosamente la principal vía de absorción. También había aumento de actividad por debajo de las capas epiteliales y la absorción transepidérmica es pues probable. Se discutieron los resultados sobre todo en la interpretación de los autorradiogramas y respecto a las deducciones para otros estudios de la absorción percutánea del paration.

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE PUBLICATION

From the Annual Review of Entomology, Vol. 5, pp. 379-404, 1960

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PESTICIDES IN RELATION TO PUBLIC HEALTH[1]

ADM-O, NCT PROGRAM
Technology Branch
Communicable Disease Center
Atlanta 22, Ga.

By Wayland J. Hayes, Jr.

Communicable Disease Center, Bureau of State Services, Public Health Service, United States Department of Health, Education, and Welfare, Savannah, Georgia

Pesticides promote health directly through the control of vector-borne diseases and indirectly through increased and improved agricultural production. On the contrary, the extensive use of any biologically active chemical implies a potential hazard which has to be evaluated.

The public health aspects of these valuable, but potentially dangerous, economic poisons have been ably reviewed, sometimes at greater length than is permitted here (6, 8, 63). The facts have changed very little. This review attempts to present some results of recent research together with enough narrative to supply continuity.

Benefits to Health

Most people at least have heard of the revolution which DDT produced in disease control. Other compounds including dieldrin, benzene hexachloride, and chlordane now have made contributions chiefly in the control of adult mosquitoes and the triatomid bug, *Thodinus prolixus* Stål, a vector of American trypanosomiasis. Lindane has been used for control of human body lice, especially where these vectors of typhus and relapsing fever have developed resistance to DDT. Diazinon [*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate] and malathion have been used for control of adult mosquitoes, and Diazinon, parathion, and malathion have been used against mosquito larvae. Most of these compounds have been used against flies; however, these materials are largely substitutes for DDT and their method of use has remained generally imitative so far as disease control is concerned. Even now, DDT is used in vector control more frequently than all other insecticides combined.

The contribution of DDT to the control of maleria, filariasis, dengue, urban yellow fever, virus encephalitis, louse-borne typhus, louse-borne relapsing fever, trench fever, plague, murine typhus, bacillary and amebic dysentery and diarrhea, leishmaniasis, bartenellosis, onchocerciasis, sandfly fever, trypansomiasis, yaws, infectious conjunctivitis, cholera, Chagas' disease, scrub typhus, scabies, rickettsialpox, tick-borne relapsing fever, Rocky Mountain spotted fever, and tularemia was the subject of a 251-page review by Simmons published in 1959 (95). The earliest relevant papers were concerned with measurements of vector control in treated and in comparable, untreated places. It required longer to collect epidemiologic evidence and, thus, demonstrate that disease control had been achieved, but this now has been done in a most impressive way. A single example (Figure 1) must suffice.

[1] The survey of the literature pertaining to this review was concluded in June 1959.

In his review, Simmons points out that malaria control, brought about by DDT, has been a factor in permitting some increase of population and a great increase in agricultural productivity in certain areas of Madagascar and India. Such a real improvement in the welfare of the community may be expected to follow improvement in the health of the members. However, this social change is more difficult to measure and document than the spleen index or parasite rate.

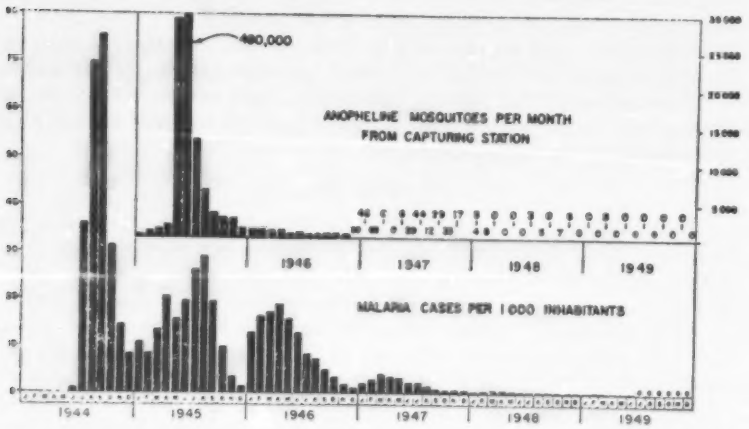

FIG. 1. Number of anopheline mosquitoes per month from capturing stations in Latina Province, Italy, and number of malaria cases per 1000 inhabitants in the same Province following spraying with DDT from June, 1945, onward [modified from Simmons (95)].

There has been such outstanding success already in disease control through the use of DDT and other chemicals that the eradication of malaria and urban yellow fever is now being attempted in many countries with the active support of the World Health Organization, the Pan American Sanitary Bureau, United Nations Children's Fund (UNICEF), International Co-operation Administration, and other international health agencies. In some countries, very much improved epidemiologic surveillance will be required if eradication is to be achieved. Drugs may be useful in the public health aspects of disease control as well as in the treatment of the individual case. However, there is still much room for improvement in vector control. This would be true even in the absence of resistance. It is much too early to evaluate the suggestion by Mathis *et al.* (74) that DDVP (2,2-dichlorovinyl dimethyl phosphate), Diazinon, malathion, and perhaps other organic phosphorus compounds which produce insecticidally effective vapors may be used for control of mosquitoes in dwellings. Many technical problems remain to be

solved. However, the first really new idea in vector control since the introduction of DDT may have been suggested.

Even in a country with agricultural surpluses, we cannot ignore the contribution which insecticides make to our food and shelter. If effective insect control and other factors did not make it possible for farmers to produce food at a higher rate than formerly, we simply would require more farmers and more cultivated land or face starvation. In spite of the progress, it is estimated that insects destroy or spoil over 10 per cent of the total crop in this country and that really thorough control might show this to be an underestimate (32). The situation in the tropics is far worse: malnutrition frequently is present, insects present a more severe problem, and less progress has been made in their control. Decker (32) has pointed out that major livestock enterprise was delayed 200 years even in our own southeastern states by uncontrolled arthropods, notably ticks and screwworm flies. It is thought that the thorough application of the best present methods would reduce agricultural losses caused by insects to one-half. The control of agricultural insects certainly must go hand-in-hand with the control of disease vectors if underdeveloped countries are to approach the achievement of health and low infant mortality now enjoyed by the countries more advanced in technology.

Production and Use

In view of the great benefits from insecticides, it is no wonder that their production has increased. It is estimated that pesticides reached a value of $274,894,750 at the manufacturer's level in 1956 (93). It is estimated that insecticides account for 36 per cent of the cost; fungicides, 32 per cent; herbicides, 13 per cent; and miticides, soil fumigants, space fumigants, and rodenticides and miscellaneous economic poisons, 8, 5, 4, and 2 per cent, respectively (41, 42, 43).

Over 120,000,000 pounds of technical DDT alone have been produced annually for several years. The annual production of synthetic organic pesticides in the United States has incerased steadily, reaching a level of 569,927,000 pounds in 1956 (93). Not only has production of a wide range of pesticides increased, but, as shown in Figure 2, this increase is expected to continue.

Another indication of the growing magnitude and variety of production is the registration of economic poisons under the Federal Insecticide, Fungicide, and Rodenticide Act as shown in Figure 3. Of the cumulative total of 90,816 registrations through June, 1958, of which 51,626 were original and 39,190 supplementary, only 8586 were cancelled voluntarily from 1953 onward (109).

To be sure, most of the registered formulations are mixtures with relatively minor variations in combinations. It has been stated (81) that only about 200 basic chemicals are registered for use in pesticide formulations and that only a small proportion of these account for the majority of the total value. Thus, it is said that there are only fifteen to eighteen major insecti-

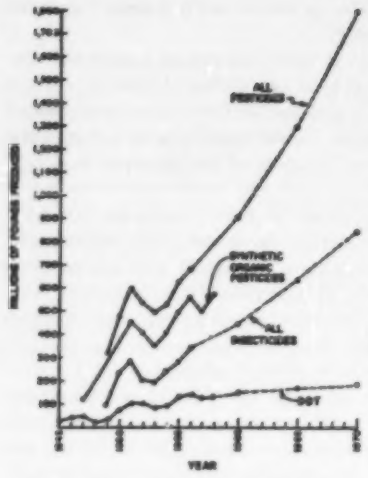

FIG. 2. United States production and predicted production of DDT, all insecticides, synthetic organic pesticides, and all pesticides. [Data from Commodity Stabilization Service, U. S. Department of Agriculture and estimates from Fisher, 1956 (41, 42, 43) and President's Materials Policy Commission, 1952.]

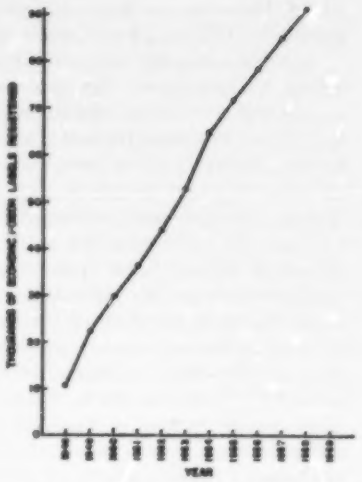

FIG. 3. Total number of economic poison labels registered under the Federal Insecticide, Fungicide, and Rodenticide Act [From Ward (109)]. Includes 51,626 original and 39,190 supplemental registrations as of June 30, 1958.

cides, ten to fifteen fungicides, four or five soil fumigants, eight to ten herbecides, and four or five rodenticides. Although this is undoubtedly true from an economic standpoint, it is necessary to take into account toxicologically all of the registered basic chemicals and a much larger number of candidate materials.

Furthermore, as new insecticides are introduced, they supplement, but do not replace, older ones. This is illustrated by Figure 4, which shows the production of two arsenicals contrasted with the production of DDT and BHC (1, 2, 3, 4, 5, 6-hexachlorocylohexane). Again, although DDT, the first synthetic insecticide introduced, is still made in greater tonnage than any other, it is estimated that the phosphorus compounds now represent between 15 and 20 per cent of the total value of insecticides produced in the United States (81).

Paralleling the increase in pesticides, there has been an increase in the number and variety of their uses and in the methods of their application. Crops are treated which formerly were left unprotected. The expansion of suburbs has been associated with increased use of pesticides by amateurs for lawns and small flower or vegetable gardens. Figure 5 shows the trend in

the number of acres of crop land in California treated by aircraft. Another relatively new method of application involves the use of low volume, high concentration sprays applied by tractor-drawn sprayers.

Magnitude of the Toxicology Problem

Some indication of the interest in the potential hazard of pesticides is indicated by the number of laboratories investigating the toxicity of these compounds. Thirty-six of these laboratories and tabular data on them have been listed by the World Health Organization (111).

The hazard of a given compound is the danger which it presents; the toxicity of a compound is its inherent ability to injure living organisms. Hazard can be measured only in relation to practical conditions, although it can be predicted, to a limited extent, from toxicity. Conversely, toxicity may be guessed from the results of accidents; but, unless the dosage involved in such accidents is known accurately, toxicity can be measured only under standardized experimental conditions. Chemical structure largely determines the absorbability, toxicity, and metabolic fate of a chemical, but the way the material is used determines whether it ever will be available for absorption in the first place. The toxicity of each compound usually varies for different species and different ages and may vary for the two sexes. The toxicity of a

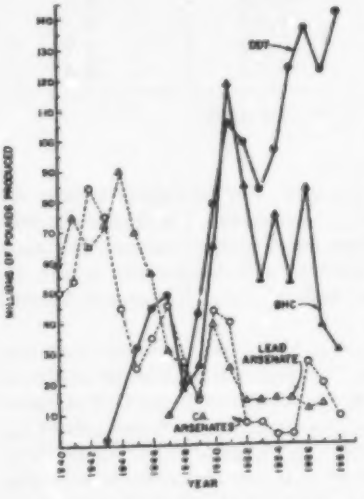

Fig. 4. United States production of two arsenical insecticides and two synthetic insecticides. (Based on U. S. Tariff Commission and Bureau of Census figures.)

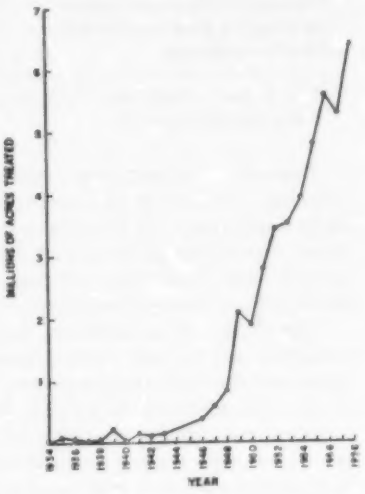

Fig. 5. Area of land in California treated with pesticides in successive years. (Based on Annual Reports, California Department of Agriculture.)

compound may be influenced by its stability and particle size, the presence of solvents or other adjuvants, the nutrition of the subject, the timing of application, and other factors. Chronic toxicity may be determined by the storage of a poison or its metabolites in the tissues, or by the accumulation of some biochemical or morphological tissue injury, or both, or by both storage and tissue injury. The hazard presented by a compound depends on all the aspects of its toxicity and also on the method of use, the degree of odor or other warning it offers, the education of those who use it or contact it indirectly, and other subtle factors.

TABLE I

DEATHS FROM SELECTED GROUPS OF CAUSES AND CRUDE DEATH RATES PER 100,000 POPULATION, UNITED STATES, 1956*

Cause	Number	Rate†
All causes	1,564,476	935.4
Malignant neoplasms	247,357	147.9
Cardiovascular-renal diseases	854,152	510.7
Alcoholism and other drug addiction	2,161	1.3
Accidents	94,780	56.7
Motor vehicle	39,628	23.7
Poisoning by gases and vapors	1,213	0.7
Poisoning by solids and liquids	1,422	0.9
All other accidents	52,517	31.3

* *U. S. Public Health Serv. Vital Statist. U. S., 1956* (1958).

† Per 100,000 population.

Hazards associated with insecticides and other pesticides include the following. (*A*) Hazards of manufacture or formulation. (*B*) Hazards of mixing or application. (*C*) Hazards to persons not directly or intentionally associated with chemicals including: (*a*) children and irresponsible adults, (*b*) persons whose food bears residues of chemicals, and (*c*) persons exposed incidentally or environmentally.

The danger from pesticides may be judged from mortality statistics, morbidity records, and special surveys. The deaths from selected groups of causes and the crude death rates per 100,000 population in the United States during 1956 are shown in Table I. Most poisoning by pesticides falls in the category of accidental poisoning by solids and liquids. Poisoning by fumigants forms only a small proportion of the category of accidental poisoning by gases and vapors. It may be seen that all kinds of fatal accidental poisoning constitute only a small proportion of deaths from accidents generally and an even smaller proportion of deaths from all causes.

Table II shows the total accidental deaths in the United States caused by chemicals for the period 1939 to 1956. It may be seen that the number of

cases attributed to solids and liquids has increased slightly during the period but there has been little change in the rate of poisoning because of population growth. There has been no increase in the rate of poisoning following the commercial introduction of DDT in 1946 and the introduction of other new materials in subsequent years. Rather, it would seem that a gradual improvement in the record still is taking place.

TABLE II

TOTAL ACCIDENTAL DEATHS IN THE UNITED STATES CAUSED BY CHEMICALS AND RATE PER 100,000 POPULATION*

Year	Gases and vapors		Solids and liquids	
	Number	Rate	Number	Rate
1939	1,396	1.1	1,371	1.0
1940	1,562	1.2	1,324	1.0
1941	1,464	1.1	1,191	0.9
1942	1,694	1.3	1,193	0.9
1943	2,038	1.5	1,254	0.9
1944	1,907	1.4	1,381	1.0
1945	2,131	1.6	1,532	1.2
1946	1,874	1.3	1,536	1.1
1947	1,938	1.4	1,504	1.0
1948	2,002	1.4	1,436	1.0
1949	1,617	1.1	1,634	1.1
1950	1,769	1.2	1,584	1.1
1951	1,627	1.1	1,497	1.0
1952	1,397	0.9	1,440	0.9
1953	1,223	0.8	1,391	0.9
1954	1,223	0.8	1,339	0.8
1955	1,163	0.7	1,431	0.9
1956	1,213	0.7	1,422	0.9

* *U. S. Public Health Serv. Vital Statist. U. S., 1939–1956* (1940–1958).

The classification "Other Acute Poisonings" used around the turn of the century essentially is comparable to a combination of the three classifications used today: "Accidental Poisoning by Solid and Liquid Substances," "Accidental Poisoning by Noxious Foodstuffs," and "Accidents Caused by Bites and Stings of Venomous Animals and Insects." In 1956, the latter three categories carried a combined rate of 0.9 per 100,000 population; the rate for the former classification for the death registration states of 1900 was 4.2 per 100,000. During the following decade, this figure rose to 5.2 in 1903 and then declined to 3.4 in 1909. As shown by Table III, the gradual decline in the number of deaths caused by the older pesticides (only two of which are

TABLE III

NUMBER OF ACCIDENTAL DEATHS IN THE UNITED STATES ATTRIBUTABLE TO SELECTED CAUSES*

Year	Arsenic and related compounds	Nux vomica and strychnine	Barbituric acid and derivatives
1939	109	82	201
1940	73	67	246
1941	70	40	232
1942	71	50	197
1943	85	54	226
1944	77	54	270
1945	58	52	392
1946	63	45	436
1947	48	37	418
1948	63	30	419
1949	57	22	466
1950	58	23	409
1951	57	16	363
1952	49	24	327
1953	52	21	337
1954	37	14	345
1955	41	17	411
1956	58	7	323

* *U. S. Public Health Serv. Vital Statist. U. S., 1939–1956* (1940–1958).

listed) is counterbalanced by an increase in accidents involving sleeping pills, for example.

A careful study of deaths attributed to accidental poisoning in the United States in 1956 has been made by Short (94). Deaths caused by solid and liquid pesticides constituted 9.8 per cent of those caused by solid and liquid substances generally. This finding is similar to that of Conley (26), who reported that 7.8 to 12.8 per cent of poisoning cases each year from 1946 through 1955 were caused by pesticides. In the United States during 1956, 152 deaths were caused by pesticides as follows: arsenic, 54; phosphorus, 21; thallium, 8; mercury, 2; nicotine, 4; strychnine, 3; rotenone, 1; camphor, 1; parathion, 11; Diazinon, 2; demeton, 1; TEPP (tetraethyl pyrophosphate), 1; unidentified organic phosphorus insecticide, 1; endrin, 3; chlordane, 3; lindane, 2; aldrin, 1; dieldrin, 1; combinations of chlorinated hydrocarbon insecticides, 2; other specified pesticides, 17; and unspecified pesticides, 13.

It is sometimes assumed that most deaths caused by lead and arsenic are associated with pesticides. This was true in 1956 for arsenic but not for lead. Of 62 presumably accidental deaths involving arsenic (including four originally not so classified), 54 were caused by economic poisons, one was caused by a mercurial medication, and seven were really suicides. On the contrary,

not a single case of lead poisoning was associated with a pesticide. Most of the lead cases were associated with paint gnawed from surfaces by infants. It was true that most deaths caused by pesticides were assigned to the category "other and unspecified solid and liquid substances;" of 144 pesticide cases attributed to solids and liquids during 1956, 86 fell in this category.

It is of interest to note that 104 of the 152 deaths caused by all pesticides in 1956 were caused by compounds introduced before DDT. Only 35 deaths definitely were associated with the new synthetic compounds, although some of the 13 other deaths caused by unidentified pesticides doubtlessly were caused by synthetics. During 1956, children up through the age of nine accounted for 94 deaths from pesticides (62 per cent); 78, or just over half of the total number, were children three years old or younger. In 1956 and other recent years also, the older compounds remained the chief cause of fatal poisoning by pesticides and children remained the chief victims.

It frequently is estimated that there are about 100 nonfatal accidents for every fatal one. The ratio may be even higher if one includes as cases those episodes where exposure is observed or suspected without the development of any characteristic symptoms. Among a total of 2407 hospitalized poisoning cases reported by the American Red Cross for one important city, the ratio of nonfatal to fatal cases varied from 25:1 to 115:1 for the separate years 1951 through 1958 and averaged 50:1. By contrast, among cases reported to poison control centers, the rate of hospitalization was less than 10 per cent, and the ratio of nonfatal to fatal cases is 750:1. Approximately 70 per cent of cases reported to poison control centers were asymptomatic (20).

Among the 2407 hospitalized cases mentioned above, the proportion of nonfatal and fatal cases associated with pesticides was 5.8 and 4.3 per cent, respectively. These percentages are of slightly lower magnitude than those given earlier for fatal cases associated with pesticides, perhaps because the values come from an urban area. In one report of 579 cases reflecting considerable rural experience (87), only the more serious cases were recorded (ratio of 13 nonfatal to 1 fatal), and the proportion of nonfatal and fatal cases caused by pesticides was 10.4 and 8.9 per cent, respectively.

A number of surveys of the incidence of poisoning have been carried out, sometimes as the result of local complaints that insecticides were the cause of illness in the persons who were exposed unintentionally and only slightly (45, 50, 60, 90, 98). In each instance, cases of acute poisoning were found among those heavily exposed and reported in the papers cited or separately. The degree of illness corresponded, as nearly as could be judged, to the degree of exposure. Cases of mild poisoning resembled severe cases except in degree. Men who had extensive occupational exposure to parathion frequently showed some depression of blood cholinesterase activity, even though they remained well. However, in no instance were insecticides found to be a cause of chronic effects or a contributing cause of asthma, influenza, or any other of a wide range of diseases which some have attributed to DDT or to pesticides generally. It has been possible to measure the excretion of *p*-nitro-

phenol in the urine of people whose only exposure to parathion resulted from their living near treated orchards (40). However, the occurrence of even mild illness always was associated with greater exposure and the excretion of the parathion derivative at a much higher rate than that encountered by these environmentally exposed persons.

Methods of Study and Clinical Results

By necessity, first estimates of the safety of a pesticide must be based on its toxicity to animals. The evaluation of chemicals which may occur in trace quantities in food is based exclusively on animal tests, analytic methods, and

TABLE IV

Relationship between Toxicity and Safety of Pesticides

Compound	LD_{50} values in male rats		Kind of exposure associated with systemic poisoning	Greatest severity of observed poisoning
	Oral	Dermal		
DDT	113	2,510	Ingestion	Severe
Lindane	88	1,000	Ingestion	Severe
Dieldrin	46	90	Ingestion and occupational	Severe
Methyl parathion	18	65	Occupational	Mild
Guthion*	13	220	Occupational	Mild
Parathion	13	21	Ingestion and occupational	Severe
Thimet†	2	6	Occupational	Severe
Phosdrin‡	6	5	Occupational	Severe

* *S*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl) *O,O*-dimethyl phosphorodithioate.

† *O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate.

‡ 2-methoxycarbonyl-1-methylvinyl dimethyl phosphate.

the results of residue measurements. The most recent and complete presentation of the tests required by the Food and Drug Administration is that of Lehman *et al.* (71). A discussion of these tests is beyond the scope of this paper, but anyone interested in the scientific aspects of the problem must consider the critical review of Barnes & Denz (7).

New residue tolerances are published in the *Federal Register*. A generally available list complete through March 1, 1959, has been published by the National Agricultural Chemicals Association (86).

There is a useful correlation between occupational hazards based on actual use experience and dermal LD_{50} values, while the correlation with oral LD_{50} values is less close. This is illustrated by Table IV from Gaines (49). The prediction of occupational poisoning may be improved somewhat by studying the effect of repeated doses of a pesticide in animals. However, ani-

mal tests serve their best purpose when some idea of the mode of action can be grasped and explored.

Because the toxicity of a chemical differs for different species, it is necessary that a final determination of danger be based on studies of man himself. Such studies may involve cases of accidental poisoning when the dosage is known, cases of occupational exposure when that exposure can be measured and clinical effects studied, and finally, the careful investigation of people who voluntarily receive measured doses according to a predetermined plan. Several insecticides now have been studied in man.

The potential hazard of DDT lies in its possible long-term effects and in the fact that it is stored, even when absorbed in small amounts. Walker *et al.* (108) found that DDT occurred in traces in all of the 18 restaurant meals which they analyzed. The average daily intake of DDT based on all meals—breakfast, lunch, and dinner—was 0.184 mg. The greatest amount of DDT which could have been eaten in the three meals of a day was 0.388 mg. The storage of DDT in human fat was demonstrated early (65, 70).

In view of these facts, Hayes and his colleagues (58) fed volunteers DDT dissolved in oil at doses of 0, 3.5, and 35 mg. per man per day. Because all the men were on an ordinary diet, these dosage levels were chosen as being about 1, 20, and 200 times the ordinary dietary level. Along with four controls, 10 men ate the DDT daily for a year and a few of them for somewhat longer. No clinical effect associated with dosage was detected either by the men themselves or by careful physical examination and laboratory testing. The storage of DDT was measured and a method for accurately measuring the excretion of DDA [bis(chlorophenyl) acetic acid] in the urine was developed (28). About one year was required for the men to approach equilibrium of DDT storage. The storage of DDT in the fat was proportional to dosage. It ranged from 3 to 13 p.p.m. (mean, 7.4 p.p.m.) for all the men at the beginning of the experiment. Following 10 or more months of dosage at 20 times the ordinary dietary level, one man stored recrystallized DDT at a concentration of 90 p.p.m., while two others stored technical DDT at concentrations of 26 and 33 p.p.m. Following the same length of dosage at 200 times the ordinary dietary level, the men stored recrystallized DDT at concentrations of 216 to 466 p.p.m. (mean, 340 p.p.m.) and technical DDT at 101 to 367 p.p.m. (mean, 234 p.p.m.). The difference between recrystallized and technical DDT was statistically significant, but the physiological basis is not fully understood. The study is being repeated with 21 months of dosage and 27 months of further observation with emphasis on measurement of DDA excretion and on the loss of DDT from storage following cessation of dosage.

In the meantime, measurement of DDT storage in the fat of groups of people with different degrees of dietary, environmental, and occupational exposure revealed that those who lived near treated fields show only a trivial increase of DDT in body fat as compared with people in the general population (61). People who eat essentially no meat store significantly less DDT (mean 2.3 p.p.m.) than the general population (mean 4.9 p.p.m.), as one

would predict from the greater average concentration of the compound in animal fat in contrast to other food (108). Recent occupational exposure to DDT in agriculture produced an average storage of 17.1 p.p.m. Heavy occupational exposure produces high storage levels. The highest value reported for man was 648 p.p.m. found in a worker in a formulating plant (58). Ortelee (88) examined 40 formulating plant workers and measured the excretion of DDA [bis(chlorophenyl)acetic acid] in their urine. The results indicated that 26 of these men had absorbed DDT at a rate equal to, or higher than, that associated with an oral dose of dissolved DDT at the rate of 35 mg. per man per day. The men had been exposed at this rate for 0.5 to 6.5 years (mean, 3.5 years) but showed no ill effects as judged by medical examinations, their own reports, or their work-attendance records.

The oral toxicities of schradan, dimefox, and parathion in man have been studied by Edson (35, 36, 38). Daily doses of schradan at the rate of 0.013 mg. per kg. per day caused moderate cholinesterase depression but no signs or symptoms. Daily doses of dimefox as great as 0.0020 mg. per kg. per day caused no physiological disturbance and no significant depression of blood cholinesterase. Daily doses at the rate of 0.0034 caused a slight but definite depression of cholinesterase without any symptomatology. In a similar way daily doses of parathion at a rate of 0.021 mg. per kg. per day produced no effect while 0.078 mg. per kg. per day produced a moderate depression of blood cholinesterase without clinical evidence of injury.

The oral toxicity of dinitrophenol was studied by Harvey *et al.* (57) who found that two of five men given 75 mg. of DNOC (4,6-dinitro-*o*-cresol) daily for five or more days developed headache, lassitude, and malaise after five and seven days. The symptoms corresponded with blood levels of 20 p.p.m. and with peaks of 40 and 48 p.p.m. In practice, it seems wise to discontinue the exposure of workers when their blood level reaches 15 p.p.m. and obligatory to do so when the blood level reaches 25 p.p.m. (37).

The toxicity of aerosols containing malathion was investigated by Golz (52). Groups of four men each received 84 one-hour exposures in 42 consecutive days at dosages of 0, 0.15, 0.6, and 2.4 gm. of malathion per 1000 cu. ft. of air. At no time did the men experience any cholinergic symptoms or signs, nor was there any significant or dose-related change in blood cholinesterase. The highest dosage, which was far greater than anything which would result from practical use, was moderately irritating to the nose and conjunctiva.

The mechanism of action of TEPP (tetraethyl pyrophosphate) on the eye was studied by Upholt *et al.* (99). It was found that instillation of two drops of a 0.1 per cent solution in peanut oil causes maximal miosis, a decrease in light perception, and an increased depth of focus in the eye. When the application was unilateral (but not when the application was to both eyes) the volunteer lost the ability to judge distance. More recently, a pilot who had been applying TEPP and was having great difficulty in landing his plane found that his vision cleared when he closed one eye, and he landed without further difficulty (90).

The expression "chronic disease" is properly reserved for sickness which persists or recurs with the result that scarring, atrophy, or other tissue injury, which the pathologist recognizes as evidence of a prolonged injury, occurs. Among the pesticides, compounds of lead, arsenic, and mercury long have been recognized as potential causes of chronic injury, although they also may produce acute damage. Many of the newer pesticides may produce illness after repeated exposure; this frequently is called chronic poisoning, but this usage misses an important distinction. To be sure, sufficient repeated dosage with any of the organic phosphorus insecticides will produce a depression of blood cholinesterase activity during exposure. However, if clinical poisoning does occur, it is acute, and recovery of blood cholinesterase or physical well-being is just as fast as in persons whose blood cholinesterase has been reduced to the same degree by a single dose. The full picture of chronic poisoning has not been demonstrated for any of the new pesticides (unless one considers the organic mercury compounds as new). However, the recurrence of convulsions has been observed in men and rats months after dermal exposure to dieldrin was discontinued. This indicates a certain chronicity, although the mechanism remains unknown (63).

Considerable attention has been paid to the potentiation between certain pairs of organic phosphorus compounds (27, 34, 46, 47, 82, 83, 84). DuBois (34) has shown that EPN (*O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate) blocks the enzymes which normally convert malathion (*O,O*-dimethyl dithiophosphate of diethyl mercaptosuccinate) to relatively harmless materials; the conversion of malathion to the toxic malaoxon is not blocked and thus the body's defense against malathion is crippled. It is supposed that potentiation between other pairs of organic phosphorus compounds depends on a similar mechanism. A case of human poisoning has not been reported in which it seems necessary to invoke potentiation in order to explain the dosage-response relationship, but the possibility of potentiation certainly has to be kept in mind in connection with occupational exposure.

Routes of Exposure

It may be nearly as valuable to know the magnitude of exposure by different routes as to know the toxicity of a compound. It has been established for some time, on the basis of animal experiments, that many of the newer pesticides, except DDT, are absorbed easily through the skin even when not in solution. This property has been associated correctly with cases of poisoning following massive skin exposure, but the importance of dermal absorption in the more ordinary cases of occupational illness frequently has been missed. This failure of association was caused partly by a natural tendency to think in terms of the older economic poisons, most of which offer a serious threat by the oral route only, or in terms of the dusts, fumes, and gases, which are seriously dangerous by the respiratory route only and which constitute major industrial hazards. Another reason for past failure to associate dermal exposure with clinical effects was a lack of appreciation of the magni-

tude of dermal exposure which spraymen and mixers commonly receive. It has been shown for parathion (9), malathion and chlorthion (29), DDT (110), and dinitro ortho-cresol (10) that the "imperceptible" dermal exposure under practical conditions of agricultural or residual spraying is from 2 to 494 times as great as the respiratory exposure, even when only the face, neck, and lower arms are exposed. If clothing is less complete, the dermal exposure will be even greater. The fine mist which spraymen encounter is often imperceptible as it falls, but in a short time it leaves an easily visible deposit on eye glasses, wrist watches, or any other impervious, polished objects worn on the face or arms. This so-called imperceptible mist is what was measured in the research cited above, and it is in addition to spillage or any other gross contamination. Because DNOC (4,6-dinitro-orth-*o*-cresol) is a brilliant yellow dye, it was possible for Edson (39) to compare the total skin contamination (whether from spillage or drift) with the blood level; he found a striking and consistent relationship both in factory workers and in spray operators. In one man, a single contamination caused a rise of blood DNOC from 6 μg. per ml. to 30 μg. per ml. during the next 24 hr. When 1 gm. of DNOC, as a slurry inside a rubber glove, was kept in contact with a human subject's hand for 8 hr. the blood DNOC level rose to 15 μg. per ml. within 24 hr. This concentration of DNOC would indicate the presence of just over one-tenth of the total applied dose in the blood.

Technical questions remain about the relationship between the degree and timing of skin exposure, the rate of absorption, and the appearance of enzyme changes or clinical illness. However, there is no doubt that the measured dermal exposure of spraymen is adequate to account for the majority of cases of occupational poisoning by many newer pesticides. On the contrary, with exceptions mentioned below, measured respiratory exposure is entirely inadequate to explain the results. Oral exposure to pesticides during ordinary spraying has not been measured, but the amount ingested in various ways, such as eating with unwashed hands, appears to be small in most instances.

The protective effect of ordinary clothing and of special devices has been measured in two studies of DDT spraying (62, 110). The clothing factor is implicit in a comparison of the results of Wolfe *et al.* (110) on men with customary exposure and the results of Fletcher *et al.* (44) on men with exceptionally complete protection. The protective effect of clothing—and thus, the importance of skin exposure—is shown by the inverse relationship between exposure and the occurrence of symptoms in men applying dieldrin indoors (63).

Workers among crops previously treated with parathion may be poisoned by the residues. An episode was reported first by Ingram (66), but recently the matter has been studied more extensively by Quinby & Lemmon (89) who reported 12 episodes involving at least 149 persons of whom 78 showed clinical poisoning. The cases were distinguished by their mildness and by the high indicence among the same groups. In most of the episodes, the residues

were relatively fresh, but in a few the residues were 12 to 33 days old. The mechanism by which some residues remained active for more than a week has yet to be explained.

Respiratory exposure may be important, especially if the exposure is in closed spaces and the compound has a high vapor pressure or is dispersed as a fine aerosol. The volatile fumigants present their hazard almost exclusively by the respiratory route. The danger of certain alkyl mercurial compounds by this route is great, though less obvious.

What has been said above about occupational poisoning is not meant to underrate the importance of oral exposure when it does occur. Most cases of poisoning in children involve ingestion.

Significance of Incidental Exposure

By incidental or environmental exposure is meant any exposure which is neither voluntary nor strictly accidental. Thus, incidental to treating a crop, some spray may fall in neighboring fields or suburbs without any intent to treat those areas and without any accident in the usual sense.

There is evidence that the release of lindane vapor indoors by vaporizers leads to the development of urticaria or other manifestations of hypersensitivity in a few people (23, 24, 72). Overheated vaporizers have produced fumes which were highly irritating to everyone who encountered them even momentarily (23).

As mentioned above, there have been complaints about incidental exposure to sprays or dusts applied to crops. With rare exceptions, crop dusts are applied in such a way that they are only temporarily air-borne. The persistence of the material in the air depends on particle size, particle density, and height of release. Dusts tend to remain suspended longer than sprays. Thus, it happens that one sometimes can see a relatively discrete cloud of dust carried for a quarter of a mile or more from the point at which it was released by an airplane. However, good agricultural practice requires that application be made when conditions (including lack of wind) permit the pesticide to settle on the crop for which it is intended.

In a study by Batchelor & Walker (9), the highest concentration of parathion found inside an orchard during spraying and dusting operations was 0.74 mg. per m.^{3}. Some investigators (18, 91, 97) have found similar or lower maximal values. The values obtained by Kay *et al.* (69) for spray and by Braid *et al.* (17) for dust were significantly higher, ranging as high as 15.0 mg. per m.^{3}. High concentrations are favored by wind or by the use of dust. Loading and mixing operations generally involve higher atmospheric concentrations than spraying and dusting operations under the same weather conditions (18, 19). Thus, Batchelor & Walker (9) found as much as 5.53 mg. per m.^{3} during loading and mixing. Windy conditions may largely eliminate the difference (69). Several studies have shown that the concentration of parathion in the air over treated fields falls to negligible levels in a short time (18, 69, 91, 97).

The highest concentration detected at a place which might involve fairly extended, nonoccupational exposure was 0.02 mg. per m.^{3} (9). Braid *et al.* (17) showed that persons 50 feet directly downwind from a dusting operation may receive a few minutes of exposure, at a rate as high as 3.6 mg. per m.^{3}, as the dust cloud drifts over them. These and the other values given above for parathion may be compared with the maximal allowable concentration of 0.1 mg. per m.^{3} per 8 hr. of exposure to the compound as established by the American Conference of Governmental Industrial Hygienists (5). Emphasis has been put on parathion because it is the most toxic economic poison used so extensively and also because it has been most studied.

Measurement has shown, as one would expect, that the atmospheric concentration of parathion (17) and other insecticides (29) decreases approximately exponentially when the insecticide is carried downwind from a point source. However, Culver and his associates (29, 30) have pointed out that a cloud of insecticide tends to spread out as it drifts so that an observer standing in the drift at a distance from a moving sprayer is exposed for a longer time than a person standing nearer the spray machine. The longer time spent in the dilute cloud may produce almost as much exposure as a shorter time in a more concentrated cloud. In any event, if a cloud of insecticide does drift, it exposes a given area only briefly. People who might otherwise receive unintentional exposure get considerable protection from the direct application of crop poisons by aircraft if they are indoors even with the doors and windows open (21, 30).

Thus, the exposure of persons who happen to be near treated fields is small and transient. By contrast, the exposure of men who actually apply pesticides is far greater in intensity and especially in duration. The surveys mentioned above indicate that crop dusting, even with the most poisonous compounds commonly used, does not present a measurable hazard to persons who are exposed incidentally, although it may present a hazard to those who do the work.

Economic poisons are applied purposely, and they differ in this way from ordinary air pollutants, the release of which is more or less accidental and even may represent the loss of a useable by-product. Incidental exposure to crop dusts is generally momentary and thus different from the usual exposure to smog. Each crop dust is a clearly defined formulation of known toxicity, while smog is a variable mixture of compounds, only some of which have been evaluated or even identified. The most hazardous route of exposure of crop workers to pesticides is dermal; the important route of exposure of all of us to smog is respiratory.

There have been no cases of illness in this country from insecticide residues on food when formulations have been used according to directions. The same is true in Britain (39). However, there have been several instances in which insecticides used quite improperly on foods have led to poisoning. Nicotine sulfate residues caused illness in 11 persons who ate mustard greens which a farmer had sprayed with double the recommended dosage and then

sold the next day. Even two weeks after spraying, the residues on this particular crop were as high as 69 to 123 p.p.m.; the official tolerance is 2 p.p.m. (73). Two families were poisoned when toxaphene sprays were applied contrary to directions to collard greens and chard (75). The residue on the collards averaged 3315 p.p.m., and 3126 p.p.m. remained after the greens were washed three times. If toxaphene is applied to such crops at all, it is supposed to be used on seedlings only in such a way that no residue is present at harvest.

Related to the improper treatment of food with pesticides is the contamination of food during shipment. In one instance, the contamination of flour by endrin during shipment led to the poisoning of 59 people who ate bread made from the flour. Much of the contamination probably came from use of the flour sacks to cover the loaves as they rose (31).

A number of people have been genuinely concerned by articles which allege that insecticides, and especially residues of them on foods, are the cause of many human and animal diseases. DDT particularly has been incriminated. Papers by a very small number of physicians (12 to 16) have continued to receive sensational publicity in a certain segment of the popular press (33, 76 to 80, 92). DDT has been claimed to be the direct cause of a virus disease (later identified as influenza) (1, 2), a psychoneurotic syndrome, and a contributing cause of poliomyelitis, hepatitis, cardiovascular disease, and cancer, as well as of several diseases of animals. Less publicized claims have attempted to relate insecticides with fungus infection, allergy, sinusitis, gastrointestinal upsets, dehydration, alkalosis, malnutrition, pneumonitis, and insanity.

It is important to realize that the alarm which has been expressed is not shared by scientists who actually have studied insecticides nor by the vast majority of physicians. During years of investigation, it has been impossible to confirm the allegation that insecticides, when properly used, are the cause of any disease either of man or animals. When misused, however, they may produce poisoning.

Some of the diseases which are claimed to have increased because of DDT, in fact, have shown no increase. Some of the other diseases are increased largely because of the changing distribution of age groups in the population of the United States. Because of improvements in the control of communicable diseases of children and adults, a greater proportion of people live to be old enough to have cancer and those forms of heart trouble which strike in middle or old age. It is true that there are a few diseases, such as lung cancer, which have shown a real, as well as a relative, increase. However, as with cancer of the lung, the trend began before the newer insecticides were used and appears to be much better explained by other factors. Furthermore, animal studies indicate that DDT is not carcinogenic (11, 56).

In the clinical studies carried out by the Public Health Service, particular attention has been paid to workers who, because of their occupational exposure, came in contact with much more DDT than people in the general popu-

lation. It is a general principle that, when large samples are considered, poisoning can be expected to appear most quickly, frequently, diversely, and severely in those persons most extensively exposed. This is a principle which alarmists appear to have ignored.

Experience in Other Countries

As suggested above, the safety record of the newer pesticides in the United States is good. The same is true in Canada and England, yet the picture is far from universal. The greatest difficulty has involved parathion under a variety of trade names. In spite of the poor reporting of cases and the relatively restricted use of this and other compounds, the majority of fatal cases of parathion poisoning are known to have occurred in underdeveloped countries. Thus, the total number of deaths caused by parathion in the United States since the commercial introduction of this agricultural chemical in 1947 is less than 100. By contrast, 96 cases of parathion poisoning with 13 deaths were reported from a small portion of the cotton-raising section of Brazil in a single year. More than 100 fatal cases were reported in India in 1958 (68). In the same year there were 74 cases, including 67 deaths, from the Province of Syria, United Arab Republic, and 20 deaths occurred from the same cause in Jordan (112). For some reason, which is not at all clear, there has been an average of over 1500 cases of parathion poisoning with 336 deaths per year in Japan for several years (48, 64). In each parathion case, illness was acute and frequently followed a single massive exposure.

The situation with dieldrin is quite different. There are a few isolated cases in which drinking of a solution or suspension has led to poisoning. However, the vast majority of reported cases have involved spraymen who worked for weeks or months before becoming sick. Furthermore, the onset of illness did not follow an accident but just routine exposure. Cases from Venezuela, Equador, Nigeria, India, and Indonesia have been reviewed (59, 63), and cases from several other countries have been recognized but not reported in publication. At least 90 persons showed full epileptoid convulsions and 11 have shown recurrence of illness several months after last exposure to the compound. The occurrence of illness among the spraymen on different projects is proportional to the intensity and duration of occupational exposure. Based on measurements of exposure during indoor spraying with DDT (110) and on toxicity tests of dieldrin in animals, it is clear that the dermal exposure of spraymen is adequate to account for observed illness. Fletcher *et al.* (44) measured the dermal exposure of spraymen in a program in which no poisoning had occurred. These men used the best available protective equipment and observed good personal hygiene under strict supervision. Measured skin exposure to dieldrin averaged 1.8 mg. per kg. per day of which an unknown part was removed by bathing. This value may be compared with approximately 20 mg. per kg. per day calculated for the same concentration of spray and more nearly average protective measures (110).

Prevention of Intoxication

We live in an age of responsibility as well as in an age of chemistry. The tremendous interest which exists in the prevention of intoxication depends on this sense of responsibility. Advances in the control of communicable disease afford an opportunity to attack other health problems, such as poisoning, which for some years have remained static or have shown only slight amelioration. Of course, responsibility in the use of chemicals has paralleled their introduction into industry and agriculture; if this had not been true, the safety record of chemicals would have deteriorated rapidly instead of improving slowly.

Measures for preventing injury by pesticides must be based on a firm knowledge of their physical, chemical, and biological properties. Once the research has been done, certain appropriate safety measures may be enforced under law while other measures and a proper attitude toward safety may be better communicated by education. It may be found that protective clothing, respirators, masks, air-conditioned cabs, special factory ventilation, or a wide variety of other devices may be applicable to a particular problem. In addition to regulations, education, and protective devices, a co-operative working relationship between those who apply pesticides and those who may receive incidental exposure as a result of the application will do much to promote the welfare of all in the community.

In the United States, economic poisons are subject to extensive state and federal regulations. In a few instances, specific poisons are subject to local regulations also. There are three main kinds of laws: (*a*) labeling laws, (*b*) laws governing residues on food, and (*c*) laws regulating use.

The federal labeling law is entitled "The Federal Insecticide, Fungicide, and Rodenticide Act of 1947" (100, 101, 104). Similar laws exist in most of the states, and these laws have been reprinted in a single collection (22). Labels offer an indirect control of use. Federal regulation of pesticide residues on foods is carried out under the "Federal Food, Drug, and Cosmetic Act" (103), as amended (106). Many states have similar laws.

The federal government does not regulate the use of a material directly since the use is always carried on locally. Only one state has a very extensive direct regulation of the use of pesticides: California requires that permission be obtained for each separate application of any of several of the more toxic compounds (19). However, this law is directed as much toward the protection of crops from the indiscriminate use of hormone-type weed killers as it is directed toward the protection of workers. Nowhere in this country are regulations for the protection of workers who apply economic poisons developed to the same high degree as that exemplified by comparable laws in the United Kingdom (54).

A number of states require the examination and licensing of commercial pest control operators or the special licensing of pilots who apply economic poisons, or both. In addition to the laws already mentioned, manufacturers

and packagers of pesticides may have to conform to laws regarding the transportation of dangerous materials (105) and laws regarding caustic poisons (102). Some local governments issue regulations to cover specific problems. For example, the City of Chicago and some other places have regulated the sale of thermal vaporizers for lindane.

Of the three kinds of regulations, the one concerned with labeling is by far the most important. This conclusion is indicated by a review of the safety record of pesticides in different countries. Thus, the United States and the United Kingdom have effective provisions for the labeling of pesticides and both have good safety records, although their regulations regarding residues and use are quite different. On the contrary, certain other countries with advanced technology—not to mention underdeveloped countries—have inadequate regulation of pesticide labels and a poor safety record. No matter whether labeling is enforced by law, as in the United States, or by less formal agreement between government and industry, as in England (39), good labeling is the most important single means of educating people in the proper procedures and precautions for using pesticides. Publications, posters, safety campaigns, and special courses are useful but not so universally applicable.

Before a label is approved under the federal law, research must be carried out and data presented which show that, if used according to directions, the pesticide will produce the biological result claimed and will be safe. Dangers are cited and precautions are clearly stated in the label before it is approved. Much research usually is necessary for label approval. Labels are written in terms of specific uses only, and, when a label is first issued, the poison may be restricted to a single use. Other uses may be permitted later if additional research justifies them in accordance with the above criteria. Good labeling also applies to the residue problem, because labels are not accepted for uses involving crops until residues have been determined, proper intervals from treatment to harvest have been set, and tolerance limits have been established.

The laws which regulate pesticides in the United States have been a great direct and indirect cause of research on the toxicology and pharmacology of these compounds. This research is a continuing process because of the new pesticides which are introduced and the new uses which are developed for existing compounds. However, in addition to this research which is required in accordance with the laws mentioned above, there is a need for research on the effect of pesticides on persons who use them in public health or agriculture or who encounter them in their environment as residues in food. Studies of this kind carried out by the Public Health Service offer a further protection to agriculturists and to the general population against injury by the ever increasing number and variety of economic poisons.

Diagnosis and Treatment

Information on the diagnosis and treatment of poisoning by pesticides is becoming more readily available. Useful recent books are those by Sollmann

(96), von Oettingen (107), and Gleason *et al.* (51). A more specialized work is the *Clinical Memoranda on Economic Poisons* (25).

A discussion of the diagnosis and treatment of poisoning by individual compounds is beyond the scope of this review. However, it should be recalled that cases of human poisoning by the following pesticides are on record: inorganic compounds (lead arsenate, arsenic trioxide, Paris green, sodium cyanide, thallium, sodium fluoride, cryolite, mercuric chloride, sodium chlorate, barium carbonate); other metallic compounds (several organic mercury compounds); botanical insecticides (pyrethrum, rotenone, nicotine); chlorinated hydrocarbon compounds (DDT, TDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane], BHC [1,2,3,4,5,6-hexachlorocyclohexane], chlordane, aldrin, dieldrin, endrin, toxaphene); fungicides (pentachlorophenol, ziram); organic phosphorus insecticides (parathion, methyl parathion, tetraethyl pyrophosphate, demeton, Diazinon, Phosdrin, mipafox, schradan, Dipterex [dimethyl(2,2,2-trichloro-1-hydroxyethyl)phosphate], DDVP [2,2-dichlorovinyl dimethyl phosphate], malathion, Thimet, Trithion [S-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate]); nitrophenyl compounds (dinitro-ortho-cresol); hormone-type herbicides [2,4-D(dichlorophenoxyacetic acid)]; rodenticides [strychnine sulfate, ANTU (alphanapthylthiourea), sodium monofluoroacetate]; fumigants (cyanide, methyl bromide, dibromoethane); and miscellaneous pesticides (lethane, naphthalene).

A new development of great interest is the use of 2-pyridinealdoxime methiodide (2-PAM), diacetyl monoxime (DAM), and related compounds for the treatment of poisoning by organic phosphorus compounds. Both 2-PAM (55, 85) and DAM (55) have received clinical trial with somewhat encouraging results. The compounds are not yet generally available, but they deserve much further study. They act by releasing cholinesterase from what would otherwise become an irreversible complex with organic phosphorus compounds. This action is entirely different from that of atropine, and the two treatments supplement one another.

A development of a different kind, which may prove useful in more cases of poisoning, is the measurement of effectiveness of removing poisons that have been swallowed. In a study of sodium salicylate, it was found that vomiting induced immediately or even 1.5 hr. after ingestion was more effective than gastric lavage in removing the poison (4). Several factors, including the fact that some people cannot be induced to vomit, indicate that gastric lavage must be used at times. However, more emphasis should be put on induced vomiting in the treatment of poisoning by mouth. No doubt there may other measures used in the treatment of poisoning which ought to be re-examined objectively.

Poison control centers have become very popular. The first was established in 1953, and there are now some 260 operating in 42 states. The centers enjoy a variety of services from the National Clearing House for Poison Control Centers, including statistical assistance and the publication of comprehensive progress reports (20). The contribution of the centers in main-

taining records of antidotes and of the ingredients of trade-name products is well recognized, so much so that one might suppose that physicians had never treated poisoning before. Probably much more important is the safety education which some of the centers have money and staff enough to carry out (3, 67). This education, carried out best by visiting nurses of the health department, promises to be very important in accelerating the improvement in the safety record of poisons.

Conclusion

The safety record of the newer compounds is good in the United States and several other technically advanced countries largely because of careful labeling. In countries where labeling is poor or where illiteracy or irresponsibility tend to vitiate labeling, hundreds of cases of human poisoning have occurred. Because of the usefulness of economic poisons in the control of vector-borne diseases and a wide range of agricultural pests, it should be our purpose to improve the safety of all pesticides, both old and new, in all countries, in order that their benefits may not be compromised. Since many new pesticidal compounds and some new uses have been introduced in the last 10 years, it is expected that many problems will require continued surveillance. The very existence of highly active compounds, some of which are stored in the tissues of a majority of people, requires prolonged investigation of those whose occupations enforce exposure many times greater than that of the general population. However, there is nothing to be gained in the long run by irresponsible statements that nothing now is known of the toxicology of the newer pesticides, or that no legal control of their use exists, or—in the absence of epidemiologic proof—that a wide variety of illnesses from which mankind has suffered for generations is now caused by intoxication by the newer economic poisons.

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